* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	FEB 28	PATDPAFULL - New display fields provide for legal status data from INPADOC
NEWS	4	FEB 28	BABS - Current-awareness alerts (SDIs) available
NEWS	5	MAR 02	GBFULL: New full-text patent database on STN
NEWS	6	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	8	MAR 22	KOREAPAT now updated monthly; patent information enhanced
NEWS	9	MAR 22	Original IDE display format returns to REGISTRY/ZREGISTRY
NEWS	10	MAR 22	PATDPASPC - New patent database available
NEWS	11	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags
NEWS	12	APR 04	EPFULL enhanced with additional patent information and new fields
NEWS	13	APR 04	EMBASE - Database reloaded and enhanced
NEWS	14	APR 18	New CAS Information Use Policies available online
NEWS	15	APR 25	Patent searching, including current-awareness alerts (SDIs), based on application date in CA/CAplus and USPATFULL/USPAT2 may be affected by a change in filing date for U.S. applications.
NEWS	16	APR 28	Improved searching of U.S. Patent Classifications for U.S. patent records in CA/CAplus
NEWS	17	MAY 23	GBFULL enhanced with patent drawing images
NEWS	18	MAY 23	REGISTRY has been enhanced with source information from CHEMCATS
NEWS	19	JUN 06	The Analysis Edition of STN Express with Discover! (Version 8.0 for Windows) now available
NEWS	20	JUN 13	RUSSIAPAT: New full-text patent database on STN
NEWS	21	JUN 13	FRFULL enhanced with patent drawing images
NEWS	22	JUN 27	MARPAT displays enhanced with expanded G-group definitions and text labels
NEWS	23	JUL 01	MEDICONF removed from STN
NEWS	24	JUL 07	STN Patent Forums to be held in July 2005
NEWS	25	JUL 13	SCISEARCH reloaded
NEWS	26	JUL 20	Powerful new interactive analysis and visualization software, STN AnaVist, now available
NEWS	27	AUG 11	Derwent World Patents Index(R) web-based training during August
NEWS	28	AUG 11	STN AnaVist workshops to be held in North America

NEWS EXPRESS JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:08:08 ON 18 AUG 2005

=> file uspatful
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 16 Aug 2005 (20050816/PD)
FILE LAST UPDATED: 17 Aug 2005 (20050817/ED)
HIGHEST GRANTED PATENT NUMBER: US6931661
HIGHEST APPLICATION PUBLICATION NUMBER: US2005177917
CA INDEXING IS CURRENT THROUGH 17 Aug 2005 (20050817/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 16 Aug 2005 (20050816/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2005

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>>  records and may be searched in standard search fields, e.g., /PN, <<<
>>>  /PK, etc.                                                         <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together        <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

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          8218 UROKINASE
       2609464 HIGH
        510321 MOLECULAR
       1305287 WEIGHT
          8218 UROKINASE
            49 HIGH MOLECULAR WEIGHT UROKINASE
                 (HIGH(W)MOLECULAR(W)WEIGHT(W)UROKINASE)
       2609464 HIGH
        510321 MOLECULAR
       1305287 WEIGHT
          8218 UROKINASE
       2647311 TYPE
         14777 PLASMINOGEN
         56489 ACTIVATOR
             1 HIGH MOLECULAR WEIGHT UROKINASE-TYPE PLASMINOGEN ACTIVATOR
                 (HIGH(W)MOLECULAR(W)WEIGHT(W)UROKINASE(W)TYPE(W)PLASMINOGEN(W)
                 ACTIVATOR)
          1659 HMW
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            11 HMW-UPA
                 (HMW(W)UPA)
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         56489 ACTIVATOR
           869 UROKINASE PLASMINOGEN ACTIVATOR
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        717098 TERMINAL
        123583 FRAGMENT
           596 AMINO TERMINAL FRAGMENT
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          2667 ATF
L2         231 L1 AND (AMINO TERMINAL FRAGMENT OR ATF)

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L3         106 L2 AND AD<MAR 01 2001

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        218626 TERMINAL/CLM
         25936 FRAGMENT/CLM
            69 AMINO TERMINAL FRAGMENT/CLM
                 ((AMINO(W)TERMINAL(W)FRAGMENT)/CLM)
L4           8 L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)

=> d l4,cbib,1-8

L4   ANSWER 1 OF 8  USPATFULL on STN
2003:285082 Adenovirus-mediated intratumoral delivery of an angiogenesis
    antagonist for the treatment of tumors.
    Li, Hong, Epinay sur Seine, FRANCE
    Lu, He, Epinay sur Seine, FRANCE
    Griscelli, Frank, Paris, FRANCE
    Opolon, Paule, Paris, FRANCE
    Soria, Claudine, Taverny, FRANCE
    Ragot, Thierry, Meudon, FRANCE
    Legrand, Yves, Paris, FRANCE
    Soria, Jeannette, Taverny, FRANCE
    Mabilat, Christelle, Corbeil Essonnes, FRANCE
    Perricaudet, Michel, Ecrosnes, FRANCE
    Yeh, Patrice, Gif sur Yvette, FRANCE
    Gencell SAS, Vitry sur Seine, FRANCE (non-U.S. corporation)
    US 6638502 B1 20031028
    WO 9849321  19981105
    APPLICATION: US 2000-403736  20000629 (9)                          <--
    WO 1998-EP2491 19980427                                            <--
    PRIORITY: US 1997-44980P  19970428 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 2 OF 8  USPATFULL on STN
2003:243800 Use of a vector comprising a nucleic acid coding for an
```

anti-angiogenic factor for treating corneal neovascularization.
Abitbol, Marc, Paris, FRANCE
US 2003170209 A1 20030911
APPLICATION: US 2003-169180 A1 20030304 (10)
WO 2000-FR3653 20001221 <--
PRIORITY: FR 1999-167780 19991230
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 8 USPATFULL on STN
2003:203309 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
US 6599881 B1 20030729
APPLICATION: US 2000-610785 20000706 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 8 USPATFULL on STN
2001:93478 **Urokinase**-type plasminogen activator receptor.
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Naples, Italy
Masucci, Maria Teresa, Naples, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, W.D., Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619
APPLICATION: US 1995-442108 19950516 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 5 OF 8 USPATFULL on STN
2000:18228 Hydrophobic u-PAR binding site.
Pessara, Ulrich, Penzberg, Germany, Federal Republic of
Weidle, Ulrich, Munchen, Germany, Federal Republic of
Konig, Bernhard, Berg, Germany, Federal Republic of
Kohnert, Ulrich, Habach, Germany, Federal Republic of
Bartke, Ilse, Bernried, Germany, Federal Republic of
Dan.o slashed. , Keld, Charlottenlund, Denmark
Ploug, Michael, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, United Kingdom
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)Cancerforskningsfonden af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 6025142 20000215
APPLICATION: US 1995-458585 19950602 (8) <--

PRIORITY: DK 1994-831 19940708
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 6 OF 8 USPATFULL on STN
1999:92646 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
US 5935930 19990810
APPLICATION: US 1998-63465 19980420 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 7 OF 8 USPATFULL on STN
1999:72457 Promoter for VEGF receptor.
Williams, Lewis T., Tiburon, CA, United States
Morishita, Kaoru, Tokyo, Japan
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5916763 19990629
APPLICATION: US 1995-556424 19951109 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 8 OF 8 USPATFULL on STN
1998:42348 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
US 5741779 19980421
APPLICATION: US 1996-644290 19960510 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l4,cbib,clm,4

L4 ANSWER 4 OF 8 USPATFULL on STN
2001:93478 **Urokinase**-type plasminogen activator receptor.
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Naples, Italy
Masucci, Maria Teresa, Naples, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, W.D., Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619

APPLICATION: US 1995-442108 19950516 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for inhibiting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PAR) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of said form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administration, to the mammal, of a substance specifically binding to said form of u-PA or its proenzyme (pro-u-PA) or to a u-PAR, the substance being administered in an amount effective to reduce the binding of said form of u-PA or its proenzyme (pro-u-PA) to the receptor, thereby inhibiting activation of plasminogen to plasmin and hence inhibiting said proteolytic activity.

2. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a modification of u-PA or its proenzyme (pro-u-PA) which has retained its capability of binding to the u-PAR, but which is not capable of converting plasminogen to plasmin, to the mammal.

3. A method according to claim 2 wherein the modification of u-PA is u-PA inhibited at its catalytically active site by an inhibitor.

4. A method according to claim 3 where the modification of u-PA is u-PA inhibited by diisopropyl fluorophosphate u-PA (DFP-u-PA).

5. A method according to claim 2 wherein the modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA).

6. A method according to claim 2 wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to u-PAR is performed by administering a substance comprising a sequence which is identical or substantially identical to Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-Asn (SEQ ID NO:32) said sequence being capable of binding to the u-PAR so as to occupy a site of the receptor to which said form of u-PA or its proenzyme (pro-u-PA) is normally bound.

7. A method according to claim 6 wherein the substance is identical to Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-Asn (SEQ ID NO:32).

8. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of a

plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a u-PAR or a u-PA-binding modification of u-PAR to the mammal so as to occupy the cell receptor-binding site of u-PA and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from binding to the cell bound receptor.

9. A method according to claim 8 wherein the modification of the u-PAR is a truncated, soluble form thereof which is able to bind to a u-PAR binding site of u-PA or its proenzyme (pro-u-PA).

10. A method according to claim 9 wherein the modification of u-PAR is a polypeptide which comprises the approximately 16 kD fragment of a u-PAR obtained by chymotryptic digestion of intact u-PAR or a subfragment of said polypeptide which is capable of binding a u-PA or its proenzyme (pro-u-PA).

11. A method according to claim 9 wherein the truncated, soluble form is coupled to specific plasminogen activator inhibitor Type 1 or Type 2.

12. A method according to claim 2 wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a modification of pro-u-PA which has retained its capability of binding to the u-PAR, but which is not capable of being converted into u-PA.

13. A method according to claim 12 wherein the modification of pro-u-PA is one in which the sequence of u-PA normally cleavable by plasmin has been changed so that the u-PA is not cleaved by plasmin.

14. A method according to claim 13 wherein the Lys^{158} has been substituted with Glu or Gly by site-directed mutagenesis.

15. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQ ID NO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions, insertions, or deletions of amino acids.

16. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQIDNO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions of amino acids.

17. The method of claim 16 in which the substitutions of amino acids, if any, were conservative substitutions.

18. The method of claim 1 wherein the substance specifically binds u-PA or pro-u-PA.

19. The method of claim 1 wherein the substance specifically binds u-PAR.

20. The method of claim 1 wherein the substance is not a modified u-PA, a modified pro-u-PA, a u-PAR, a modified u-PAR, an antibody, a hormone, a growth factor, or a cytokine.

21. A method according to claim 2 wherein the modification of u-PA is u-PA inhibited at its catalytically active site by reaction with an inactivator which forms a covalent bond to an essential moiety necessary for the catalytic function of u-PA.

22. The method of claim 1 wherein the substance is a peptide.

23. The method of claim 1 wherein the substance is a polypeptide or

protein.

24. A method of inhibiting the binding of **urokinase** type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase plasminogen-activator** receptor (u-PAR) which comprises contacting a plasminogen-converting, receptor-binding form of u-PA or pro-u-PA, or a receptor (u-PAR) for said form of u-PA, with a substance specifically binding to said form of u-PA or pro-u-PA, or to a receptor (u-PAR), the substance being provided in an amount effective to inhibit the binding of said form of u-PA or pro-u-PA to u-PAR.

25. The method of claim 24 wherein such contacting occurs in the body of a mammal, as a result of the administration of the substance to such mammal.

26. A method of inhibiting the conversion of plasminogen to plasmin which comprises inhibiting the binding of a plasminogen-converting, **urokinase-plasminogen-activator** receptor by the method of claim 24, and hence inhibiting the consequent conversion of plasminogen to plasmin.

27. The method of claim 1 in which the substance is capable of inhibiting binding of u-PA or pro-u-PA to u-PAR in a medium containing 10% fetal calf serum.

28. The method of claim 27 in which the substance is capable of inhibiting binding of u-PA or pro-**UPA** to u-PAR in a supernatant of HT-1080 cells.

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=> d his

     (FILE 'HOME' ENTERED AT 14:08:08 ON 18 AUG 2005)

     FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1          9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2           231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3           106 S L2 AND AD<MAR 01 2001
L4             8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)

=> d l4,cbib,clm,1

L4   ANSWER 1 OF 8  USPATFULL on STN
2003:285082 Adenovirus-mediated intratumoral delivery of an angiogenesis
    antagonist for the treatment of tumors.
    Li, Hong, Epinay sur Seine, FRANCE
    Lu, He, Epinay sur Seine, FRANCE
    Griscelli, Frank, Paris, FRANCE
    Opolon, Paule, Paris, FRANCE
    Soria, Claudine, Taverny, FRANCE
    Ragot, Thierry, Meudon, FRANCE
    Legrand, Yves, Paris, FRANCE
    Soria, Jeannette, Taverny, FRANCE
    Mabilat, Christelle, Corbeil Essonnes, FRANCE
    Perricaudet, Michel, Ecrosnes, FRANCE
    Yeh, Patrice, Gif sur Yvette, FRANCE
    Gencell SAS, Vitry sur Seine, FRANCE (non-U.S. corporation)
    US 6638502 B1 20031028
    WO 9849321  19981105
    APPLICATION: US 2000-403736  20000629 (9)                        <--
    WO 1998-EP2491 19980427                                          <--
    PRIORITY: US 1997-44980P  19970428 (60)
```

DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for inhibiting the growth or metastasis or both of a tumor comprising introducing a vector into the tumor, the vector comprising a nucleic acid sequence encoding an **amino terminal fragment** of **urokinase** that comprises an EGF-like domain, with the exception that the nucleic acid sequence does not encode full length **urokinase**, wherein the nucleic acid sequence is operably associated with an expression control sequence that provides for expression in a cell of the tumor.

2. The method of claim 1 wherein the expression control sequence comprises a CMV promoter.

3. A method for inhibiting the growth of a tumor comprising introducing into the tumor a defective adenovirus vector comprising a DNA sequence encoding an anti-angiogenic factor operably associated with an expression control sequence that provides for expression of the anti-angiogenic factor in a cell of the tumor, wherein the anti-angiogenic factor comprises an **amino terminal fragment** of **urokinase** that comprises an EGF-like domain, with the exception that the anti-angiogenic factor is not full length **urokinase**.

4. The method of claim 3, wherein the expression control sequence comprises a CMV promoter.

5. The method of claim 3, wherein the adenovirus vector contains a deletion in the E1 region.

6. The method of claim 5, wherein the expression control sequence comprises a CMV promoter.

7. A defective adenovirus vector comprising a gene encoding an anti-angiogenic factor operably associated with an expression control sequence, wherein the anti-angiogenic factor comprises an **amino terminal fragment** of **urokinase** comprising an EGF-like domain, with the exception that the anti-angiogenic factor is not full length **urokinase**.

8. The vector of claim 7, wherein the expression control sequence comprises a CMV promoter.

9. The vector of claim 7, wherein the adenovirus vector contains a deletion in the E1 region.

10. The vector of claim 9, wherein the expression control sequence comprises a CMV promoter.

11. A pharmaceutical composition comprising the defective adenovirus vector according to claim 7 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition comprising the defective adenovirus vector according to claim 8 and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition comprising the defective adenovirus vector according to claim 9 and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising the defective adenovirus vector according to claim 10 and a pharmaceutically acceptable carrier.

=> d l4,cbib,clm,1-8

L4 ANSWER 1 OF 8 USPATFULL on STN
2003:285082 Adenovirus-mediated intratumoral delivery of an angiogenesis antagonist for the treatment of tumors.
Li, Hong, Epinay sur Seine, FRANCE
Lu, He, Epinay sur Seine, FRANCE
Griscelli, Frank, Paris, FRANCE
Opolon, Paule, Paris, FRANCE
Soria, Claudine, Taverny, FRANCE
Ragot, Thierry, Meudon, FRANCE
Legrand, Yves, Paris, FRANCE
Soria, Jeannette, Taverny, FRANCE
Mabilat, Christelle, Corbeil Essonnes, FRANCE
Perricaudet, Michel, Ecrosnes, FRANCE
Yeh, Patrice, Gif sur Yvette, FRANCE
Gencell SAS, Vitry sur Seine, FRANCE (non-U.S. corporation)
US 6638502 B1 20031028
WO 9849321 19981105
APPLICATION: US 2000-403736 20000629 (9) <--
WO 1998-EP2491 19980427 <--
PRIORITY: US 1997-44980P 19970428 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for inhibiting the growth or metastasis or both of a tumor comprising introducing a vector into the tumor, the vector comprising a nucleic acid sequence encoding an **amino terminal fragment** of **urokinase** that comprises an EGF-like domain, with the exception that the nucleic acid sequence does not encode full length **urokinase**, wherein the nucleic acid sequence is operably associated with an expression control sequence that provides for expression in a cell of the tumor.

2. The method of claim 1 wherein the expression control sequence comprises a CMV promoter.

3. A method for inhibiting the growth of a tumor comprising introducing into the tumor a defective adenovirus vector comprising a DNA sequence encoding an anti-angiogenic factor operably associated with an expression control sequence that provides for expression of the anti-angiogenic factor in a cell of the tumor, wherein the anti-angiogenic factor comprises an **amino terminal fragment** of **urokinase** that comprises an EGF-like domain, with the exception that the anti-angiogenic factor is not full length **urokinase**.

4. The method of claim 3, wherein the expression control sequence comprises a CMV promoter.

5. The method of claim 3, wherein the adenovirus vector contains a deletion in the E1 region.

6. The method of claim 5, wherein the expression control sequence comprises a CMV promoter.

7. A defective adenovirus vector comprising a gene encoding an anti-angiogenic factor operably associated with an expression control sequence, wherein the anti-angiogenic factor comprises an **amino terminal fragment** of **urokinase** comprising an EGF-like domain, with the exception that the anti-angiogenic factor is not full length **urokinase**.

8. The vector of claim 7, wherein the expression control sequence

comprises a CMV promoter.

9. The vector of claim 7, wherein the adenovirus vector contains a deletion in the E1 region.

10. The vector of claim 9, wherein the expression control sequence comprises a CMV promoter.

11. A pharmaceutical composition comprising the defective adenovirus vector according to claim 7 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition comprising the defective adenovirus vector according to claim 8 and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition comprising the defective adenovirus vector according to claim 9 and a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising the defective adenovirus vector according to claim 10 and a pharmaceutically acceptable carrier.

L4 ANSWER 2 OF 8 USPATFULL on STN
2003:243800 Use of a vector comprising a nucleic acid coding for an anti-angiogenic factor for treating corneal neovascularization.
Abitbol, Marc, Paris, FRANCE
US 2003170209 A1 20030911
APPLICATION: US 2003-169180 A1 20030304 (10)
WO 2000-FR3653 20001221 <--
PRIORITY: FR 1999-167780 19991230
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. The use of a vector comprising a nucleic acid encoding an anti-angiogenic factor for the preparation of a pharmaceutical composition intended to be administered by impregnation of a soft lens and application of said lens on to the cornea, for the prevention, improvement and/or treatment of corneal neovascularization.

2. The use as claimed in claim 1, characterized in that the nucleic acid encoding the anti-angiogenic is a nucleic acid encoding a polypeptide chosen from the N-terminal fragment of the plasminogen activator **uPA** (**ATF**), angiostatin, endostatin, the 16 kDa fragment of prolactin or platelet factor 4 (PF-4) or a combination of nucleic acids encoding at least two of these factors.

3. The use as claimed in claim 2, characterized in that the anti-angiogenic factor is chosen from the N-terminal fragment of the plasminogen activator **uPA** (**ATF**) and angiostatin K3.

4. The use as claimed in one of claims 1 to 3, characterized in that the vector is a plasmid, a cosmid or any DNA not encapsidated by a virus.

5. The use as claimed in one of claims 1 to 3, characterized in that the vector is a recombinant virus, preferably derived from an adenovirus, a retrovirus, a lentivirus, a herpesvirus or an adeno-associated virus.

6. The use as claimed in claim 5, characterized in that the recombinant virus is a defective recombinant adenovirus.

7. The use as claimed in claim 5 or 6 for preparing a pharmaceutical composition intended for administration by intraocular instillation, comprising from 1×10^{6} and 1×10^{12} pfu/ml and preferably between 1×10^{8} and 1×10^{10} pfu/ml.

8. A process for preparing a medicinal product which is useful for the prevention, improvement and/or treatment of corneal neovascularization, characterized in that a recombinant vector comprising a nucleic acid encoding an anti-angiogenic factor is mixed with one or more compatible and pharmaceutically acceptable adjuvants.

9. A pharmaceutical composition comprising a defective recombinant vector which comprises at least one nucleic acid encoding an anti-angiogenic factor, characterized in that it is formulated for intraocular administration.

10. The pharmaceutical composition as claimed in claim 9, characterized in that it comprises from 1×10^{6} and 1×10^{12} pfu/ml and preferably between 1×10^{8} and 1×10^{10} pfu/ml.

11. A kit comprising a recipient which contains a composition as claimed in either of claims 9 and 10 and at least one contact lens.

12. The use of the kit as claimed in claim 11 for the preparation of a medicinal product intended to be administered via impregnation of a lens, for the prevention, improvement and/or treatment of corneal neovascularization.

L4 ANSWER 3 OF 8 USPATFULL on STN
2003:203309 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
US 6599881 B1 20030729
APPLICATION: US 2000-610785 20000706 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for treating a thrombotic disorder that is not the result of an endotoxin-initiated coagulation cascade in a subject comprising administration of a bactericidal/permeability-increasing protein (BPI) protein product to the subject wherein said thrombotic disorder is an acute vascular disease selected from the group consisting of myocardial infarction, stroke, peripheral arterial occlusion, deep vein thrombosis, and pulmonary embolism and wherein the BPI protein product is administered in an amount effective to slow clot formation enhance clot dissolution.

2. A method for treating a thrombotic disorder that is not the result of an endotoxin-initiated coagulation cascade in a subject comprising administration of a bactericidal/permeability-increasing protein (BPI) protein product and a thrombolytic agent to the subject wherein said thrombotic disorder is an acute vascular disease selected from the group consisting of myocardial infarction, stroke, peripheral arterial occlusion, deep vein thrombosis, and pulmonary embolism and wherein the BPI protein produce is administered in an amount effective to slow clot formation or enhance clot dissolution.

3. The method of claim 2 wherein the amount of the thrombolytic agent is less than that required for a desired pharmaceutical effect when the thrombolytic agent is administered as a monotherapy.

4. The method of claims 1 and 2 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

5. The method of claims 1 and 2 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

6. The method of claims 1 and 2 wherein the BPI protein product is $rBPI_{21}$.

L4 ANSWER 4 OF 8 USPATFULL on STN
2001:93478 **Urokinase**-type plasminogen activator receptor.
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Naples, Italy
Masucci, Maria Teresa, Naples, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, W.D., Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619
APPLICATION: US 1995-442108 19950516 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for inhibiting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PAR) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of said form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administration, to the mammal, of a substance specifically binding to said form of u-PA or its proenzyme (pro-u-PA) or to a u-PAR, the substance being administered in an amount effective to reduce the binding of said form of u-PA or its proenzyme (pro-u-PA) to the receptor, thereby inhibiting activation of plasminogen to plasmin and hence inhibiting said proteolytic activity.

2. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a modification of u-PA or its proenzyme (pro-u-PA) which has retained its capability of binding to the u-PAR, but which is not capable of converting plasminogen

to plasmin, to the mammal.

3. A method according to claim 2 wherein the modification of u-PA is u-PA inhibited at its catalytically active site by an inhibitor.

4. A method according to claim 3 where the modification of u-PA is u-PA inhibited by diisopropyl fluorophosphate u-PA (DFP-u-PA).

5. A method according to claim 2 wherein the modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA).

6. A method according to claim 2 wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to u-PAR is performed by administering a substance comprising a sequence which is identical or substantially identical to Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-Asn (SEQ ID NO:32) said sequence being capable of binding to the u-PAR so as to occupy a site of the receptor to which said form of u-PA or its proenzyme (pro-u-PA) is normally bound.

7. A method according to claim 6 wherein the substance is identical to Asp-Cys-Leu-Asn-Gly-Gly-Thr-Cys-Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-Cys-Asn (SEQ ID NO:32).

8. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting plasminogen into plasmin, wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a u-PAR or a u-PA-binding modification of u-PAR to the mammal so as to occupy the cell receptor-binding site of u-PA and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from binding to the cell bound receptor.

9. A method according to claim 8 wherein the modification of the u-PAR is a truncated, soluble form thereof which is able to bind to a u-PAR binding site of u-PA or its proenzyme (pro-u-PA).

10. A method according to claim 9 wherein the modification of u-PAR is a polypeptide which comprises the approximately 16 kD fragment of a u-PAR obtained by chymotryptic digestion of intact u-PAR or a subfragment of said polypeptide which is capable of binding a u-PA or its proenzyme (pro-u-PA).

11. A method according to claim 9 wherein the truncated, soluble form is coupled to specific plasminogen activator inhibitor Type 1 or Type 2.

12. A method according to claim 2 wherein the prevention of the binding of a plasminogen converting receptor binding form of u-PA or its proenzyme (pro-u-PA) to a u-PAR is performed by administering a modification of pro-u-PA which has retained its capability of binding to the u-PAR, but which is not capable of being converted into u-PA.

13. A method according to claim 12 wherein the modification of pro-u-PA is one in which the sequence of u-PA normally cleavable by plasmin has been changed so that the u-PA is not cleaved by plasmin.

14. A method according to claim 13 wherein the Lys^{158} has been

substituted with Glu or Gly by site-directed mutagenesis.

15. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQ ID NO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions, insertions, or deletions of amino acids.

16. The method of claim 2 in which the substance comprises an amino acid sequence which (a) is identical to SEQIDNO:32, or (b) differs from SEQ ID NO:32 by not more than five substitutions of amino acids.

17. The method of claim 16 in which the substitutions of amino acids, if any, were conservative substitutions.

18. The method of claim 1 wherein the substance specifically binds u-PA or pro-u-PA.

19. The method of claim 1 wherein the substance specifically binds u-PAR.

20. The method of claim 1 wherein the substance is not a modified u-PA, a modified pro-u-PA, a u-PAR, a modified u-PAR, an antibody, a hormone, a growth factor, or a cytokine.

21. A method according to claim 2 wherein the modification of u-PA is u-PA inhibited at its catalytically active site by reaction with an inactivator which forms a covalent bond to an essential moiety necessary for the catalytic function of u-PA.

22. The method of claim 1 wherein the substance is a peptide.

23. The method of claim 1 wherein the substance is a polypeptide or protein.

24. A method of inhibiting the binding of **urokinase** type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase plasminogen-activator** receptor (u-PAR) which comprises contacting a plasminogen-converting, receptor-binding form of u-PA or pro-u-PA, or a receptor (u-PAR) for said form of u-PA, with a substance specifically binding to said form of u-PA or pro-u-PA, or to a receptor (u-PAR), the substance being provided in an amount effective to inhibit the binding of said form of u-PA or pro-u-PA to u-PAR.

25. The method of claim 24 wherein such contacting occurs in the body of a mammal, as a result of the administration of the substance to such mammal.

26. A method of inhibiting the conversion of plasminogen to plasmin which comprises inhibiting the binding of a plasminogen-converting, **urokinase-plasminogen-activator** receptor by the method of claim 24, and hence inhibiting the consequent conversion of plasminogen to plasmin.

27. The method of claim 1 in which the substance is capable of inhibiting binding of u-PA or pro-u-PA to u-PAR in a medium containing 10% fetal calf serum.

28. The method of claim 27 in which the substance is capable of inhibiting binding of u-PA or pro-**UPA** to u-PAR in a supernatant of HT-1080 cells.

L4 ANSWER 5 OF 8 USPATFULL on STN

2000:18228 Hydrophobic u-PAR binding site.

Pessara, Ulrich, Penzberg, Germany, Federal Republic of
Weidle, Ulrich, Munchen, Germany, Federal Republic of
Konig, Bernhard, Berg, Germany, Federal Republic of
Kohnert, Ulrich, Habach, Germany, Federal Republic of
Bartke, Ilse, Bernried, Germany, Federal Republic of
Dan.o slashed. , Keld, Charlottenlund, Denmark
Ploug, Michael, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, United Kingdom
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)Cancerforskningsfonden af 1989, Copenhagen K, Denmark (non-U.S. corporation)

US 6025142 20000215
APPLICATION: US 1995-458585 19950602 (8) <--
PRIORITY: DK 1994-831 19940708
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. An antibody or an antigen-binding fragment thereof which binds u-PAR at an epitope located outside residues 1-87 of mature u-PAR, and which inhibits the binding between u-PA and u-PAR.

2. The antibody or fragment of claim 1 which binds u-PAR, and which inhibits the binding between u-PA and u-PAR to an extent of at least 90% in an assay comprising incubating 100 µl of substantially u-PA free U937 cells in PBS containing 0.1% BSA with 100 µl of the antibody or fragment (20 µg/ml) for 30 minutes at 4° C., adding 100 µl ^{125} I-labelled 0.9 nM autodigested **amino terminal fragment** (**ATF-uPA**) of u-PA, consisting of residues 6-135 of mature u-PA, and incubating for 1 hour with mixing, and washing the cells 3 times in PBS containing 0.1% BSA and determining bound **ATF-uPA** by gamma counting.

3. An antibody according to claim 2, which further exhibits a substantial binding to a fragment of u-PAR in an immunoprecipitation assay, said fragment (residues 88 to end) being the C-terminal fragment of mature u-PAR obtained by incubating 750 µg purified soluble u-PAR with 100 ng α-chymotrypsin for 4 h at 37° C. in 1M NH_4HCO_3 followed by addition of 1 mM phenylmethylsulfonyl fluoride and subsequent purification of the fragment by size exclusion chromatography and immuno-affinity chromatography.

4. An antibody according to claim 2, which binds to the same epitope on u-PAR as a monoclonal antibody produced by the hybridoma cell line 1.H2.10A3 which was deposited on Jul. 7, 1994 at Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM) with accession number DSM ACC2178 under the terms and conditions of the Budapest Treaty, or as a monoclonal antibody produced by a hybridoma cell line 1.C3.26A3 which was deposited on Jul. 7, 1994 at Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM) with accession number DSM ACC2179 under the terms and conditions of the Budapest Treaty.

5. An antibody according to claim 4, which is a non-competitive inhibitor of the binding of u-PA to u-PAR.

6. An antibody according to claim 2, which is a monoclonal antibody.

7. An antibody according to claim 6, which is the monoclonal antibody produced by the hybridoma cell line 1.C8.26A3 which was deposited on Jul. 7, 1994 at DSM with the accession number DSM ACC2179 under the terms and conditions of the Budapest Treaty, or an active fragment thereof.

8. An antibody according to claim 7, wherein the equivalent is selected from the group consisting of fragments of antibodies, such as FV, $(FV)_2$, Fab, Fab', $F(ab)_2$, chimeric antibodies, humanized or human antibodies, short-chain antibody fragments containing only the CDR regions or parts thereof, and bispecific antibodies.

9. An antibody according to claim 6, which is the monoclonal antibody produced by the hybridoma cell line 1.H2.10A3 which was deposited on Jul. 7, 1994 at DSM with the accession number DSM ACC2178 under the terms and conditions of the Budapest Treaty, or an active fragment thereof.

10. A method for detecting or quantifying u-PAR which comprises forming a complex of u-PAR and the antibody of claim 2, and detecting or quantifying said complex, directly or indirectly, independently of whether the u-PAR has bound any u-PA or not.

11. The antibody or fragment of claim 1 which inhibits **uPA**-mediated plasminogen activation at the surface of U937 cells.

L4 ANSWER 6 OF 8 USPATFULL on STN
1999:92646 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
US 5935930 19990810
APPLICATION: US 1998-63465 19980420 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for treating a subject suffering from a thrombotic disorder selected from the group consisting of a thrombosis, coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral arterial thrombosis, venous thrombosis, pulmonary embolism, and thrombosis and coagulopathies associated with exposure of the subject's blood to a foreign or injured tissue surface, said disorders not resulting from an endotoxin-initiated coagulation cascade, comprising administering to said subject an amount of a BPI protein product effective to slow clot formation or enhance clot dissolution.

2. A method for treating subject suffering from a thrombotic disorder selected from the group consisting of arterial thrombosis, coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral arterial thrombosis, venous thrombosis, pulmonary embolism, and thrombosis and coagulopathies associated with exposure of the subject's blood to a foreign or injured tissue surface, said disorders not resulting from an endotoxin-initiated coagulation cascade, comprising co-administration of a pharmaceutically effective amount of a BPI protein product and a thrombolytic agent to said subject.

3. The method of claim 2 wherein the amount of the thrombolytic agent is less than that required for a desired pharmaceutical effect when the thrombolytic agent is administered as a monotherapy.

4. A method for enhancing reperfusion or reducing reocclusion in a subject treated with a thrombolytic agent comprising co-administration of a pharmaceutically effective amount of a BPI protein product and the thrombolytic agent.

5. A method for decreasing the dose of a thrombolytic agent required to establish reperfusion or to reduce reocclusion in a subject comprising co-administration of a BPI protein product and a thrombolytic agent, the

dosage of the thrombolytic agent being less than that required for a desired pharmaceutical effect when the thrombolytic agent is administered as a monotherapy.

6. The method of any one of claims 1 through 5 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

7. The method of any one of claims 1 through 5 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

8. The method of any one of claims 1 through 5 wherein the BPI protein product is $rBPI_{21}$.

L4 ANSWER 7 OF 8 USPATFULL on STN
1999:72457 Promoter for VEGF receptor.
Williams, Lewis T., Tiburon, CA, United States
Morishita, Kaoru, Tokyo, Japan
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5916763 19990629
APPLICATION: US 1995-556424 19951109 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An isolated nucleic acid molecule comprising a VEGF receptor promoter region, wherein said promoter region is contained in SEQ ID NO.1.

2. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a CREB/**ATF** element, at least one ETS-1 binding site and a nucleic acid sequence comprising residues 447-1479 of SEQ ID No.1.

3. An expression vector comprising the nucleic acid molecule of claim 1.

4. The vector of claim 3, wherein said vector is an expression vector operably linked to a heterologous gene encoding a gene product.

5. The vector of claim 4, wherein said heterologous gene is a reporter gene.

6. The vector of claim 4, wherein said heterologous gene is selected from the group consisting of IL-2, TNFα, tissue plasminogen activator, an antisense RNA corresponding to a gene encoding for a VEGF receptor or a fragment thereof, and a functionally defective VEGF receptor.

7. An isolated host cell transformed with the vector of claim 4.

8. The host cell of claim 7, wherein said host cell is a prokaryotic cell.

9. The host cell of claim 7, wherein said host cell is a eukaryotic cell.

10. The host cell of claim 9 which is a mammalian cell.

11. The host cell of claim 10 which is a human cell.

12. The host cell of claim 11 which is an endothelial cell.

13. The host cell of claim 12 which is a tumor cell.

14. An isolated host cell transformed with the vector of claim 3.

15. An isolated host cell transformed with the vector of claim 5.

16. A nucleic acid molecule comprising the sequence shown in SEQ ID NO.1, or a fragment of said sequence shown in SEQ ID NO.1, said fragment comprising at least about 500 nucleotides, said nucleic acid sequence exhibiting promoter activity.

17. An expression vector comprising the nucleic acid sequence of SEQ ID NO:1 or a fragment thereof, said nucleic acid sequence exhibiting promoter activity.

18. A process for producing a host cell containing a heterologous gene operably linked to the promoter of claim 1 which comprises: (a) transfecting a cell with an expression vector comprising said heterologous gene operably linked to the promoter of claim 1; (b) simultaneously transfecting said cell with a selectable marker gene; and (c) selecting transformed host cells on the basis of said transformed cells containing the selectable marker gene to produce said host cell containing a heterologous gene operably linked to the promoter of claim 1.

19. A method for producing a gene product comprising culturing the host cells of claim 7 under conditions wherein said gene product is produced and recovering the gene product from the cell culture.

20. The method of claim 19, wherein said host cell is an endothelial cell.

L4 ANSWER 8 OF 8 USPATFULL on STN
1998:42348 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
US 5741779 19980421
APPLICATION: US 1996-644290 19960510 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for slowing clot formation in blood comprising administering to a subject a BPI protein product in an amount effective to delay or prevent clot formation in the blood.

2. A method for enhancing clot dissolution in blood comprising administering to a subject a BPI protein product in an amount effective to enhance clot dissolution in the blood.

3. A method of slowing clot formation in blood comprising contacting the blood with an amount of BPI protein product effective to delay or prevent clot formation in the blood.

4. A method for enhancing clot dissolution in blood comprising contacting the blood with an amount of BPI protein product effective to dissolve or lyse the clot.

5. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

6. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

7. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{21}$.

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=> logoff hold
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      54.88      55.09

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 14:20:27 ON 18 AUG 2005

 * * * * * * RECONNECTED TO STN INTERNATIONAL * * * * * *
SESSION RESUMED IN FILE 'USPATFULL' AT 15:00:47 ON 18 AUG 2005
FILE 'USPATFULL' ENTERED AT 15:00:47 ON 18 AUG 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      54.88      55.09

=> d his

     (FILE 'HOME' ENTERED AT 14:08:08 ON 18 AUG 2005)

     FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1          9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2           231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3           106 S L2 AND AD<MAR 01 2001
L4             8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)

=> s l3 and powder
         389552 POWDER
L5           25 L3 AND POWDER

=> s l5 and (lecithin or methylcellulose or mannitol or lactose)
          35868 LECITHIN
          38206 METHYLCELLULOSE
          58838 MANNITOL
         101549 LACTOSE
L6           19 L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)

=> s l4 and l6
L7            1 L4 AND L6

=> d l7,cbib

L7   ANSWER 1 OF 1  USPATFULL on STN
2001:93478 Urokinase-type plasminogen activator receptor.
    Dan.o slashed. , Keld, Charlottenlund, Denmark
    Blasi, Francesco, Charlottenlund, Denmark
    Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
    Cubellis, Maria Vittoria, Naples, Italy
    Masucci, Maria Teresa, Naples, Italy
    Appella, Ettore, Chevy Chase, MD, United States
    Schleuning, W.D., Berlin, Germany, Federal Republic of
    Behrendt, Niels, Bagsv.ae butted.rd, Denmark
    R.o slashed.nne, Ebbe, Copenhagen, Denmark
    Kristensen, Peter, Copenhagen, Denmark
    Pollanen, Jari, Espoo, Finland
    Salonen, Eeva-Marjatta, Espoo, Finland
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Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619
APPLICATION: US 1995-442108 19950516 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l7,kwic

L7 ANSWER 1 OF 1 USPATFULL on STN
TI **Urokinase**-type plasminogen activator receptor
AI US 1995-442108 19950516 (8) <--
AB Activation of plasminogen to plasmin is inhibited by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator to a **urokinase**-type plasminogen activator receptor in a mammal, thereby preventing the **urokinase**-type plasminogen activator from converting plasminogen into plasmin. DNA fragments which encode for soluble, active fragments of the **urokinase**-type plasminogen activator receptor are provided.
SUMM . . . the method comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator (in the following termed u-PA) to a u-PA receptor in the mammal and thereby preventing the u-PA from. . .
SUMM According to the literature, **urokinase**-type plasminogen activator (u-PA) has been found in all mammalian species so far investigated. Several findings relate u-PA to tissue degradation. . .
SUMM . . . in the primary structure is remote from the catalytic site. The receptor binding domain is located in the 15 kD **amino-terminal fragment** (**ATF**, residues 1-135) of the u-PA molecule, more precisely within the cysteine-rich region termed the growth factor region as this region. . .
SUMM . . . al., 1985, Vassalli et al., 1985, Nielsen et al., 1988). Fragments of u-PA containing only the receptor binding domain, e.g. **ATF**, ensure specificity of the binding to the receptor, since other molecules that might bind u-PA (protease nexin and the specific. . .
SUMM . . . al., 1989, binding of the added single-chain u-PA to the receptor was prevented by preincubation of the cells with the **amino-terminal fragment** of u-PA. These experiments do not, therefore, as do the following examples, demonstrate displacement of endogenously produced u-PA, a prerequisite. . .
SUMM Human tumor cells are very commonly found to secrete plasminogen activator of the **urokinase** type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen. . .
SUMM . . . streptavidin-fluorescin isothiocyanate. The method is very sensitive, and its specificity can readily be tested by competition experiments (e.g. with the **amino-terminal fragment** of u-PA (**ATF**), t-PA, EGF, etc.).
SUMM While the present specification and claims relate predominantly to the **urokinase** type plasminogen activator (u-PA), it is obvious that the same approach can and should be used for tissue-type plasminogen activator. . .
SUMM . . . that the u-PA contains the u-PAR binding site. The receptor

binding form of u-PA can thus be pro-u-PA, u-PA, an **amino-terminal fragment** of u-PA, a u-PA that is irreversibly inhibited by e.g. diisopropyl fluorophosphate (DFP), p-nitrophenyl-p'-guanidinobenzoate (NPGB), or any other inhibitor or. . .

SUMM The enzyme **urokinase**-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg^{560}, plasminogen is activated to the. . .

SUMM Another useful modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA) (cf. Stoppelli et al., 1985).

SUMM . . . mg such as about 150 mg for an average adult person. The same considerations apply with respect to NPGB-u-PA, the **amino-terminal fragment** of u-PA, and pro-u-PA that is modified so that it cannot be cleaved by plasmin. Evidently, the higher the affinity. . .

SUMM . . . of substrates which are useful in the present method as substrates for the enzymes mentioned above are H_2 O_2, p-nitrophenylphosphate, **lactose**, urea, β-D-glucose, CO_2, RNA, starch, or malate. The substrate may be combined with, e.g. a chromophore which is either a. . .

DRWD . . . of 100 nM: bovine serum albumin (lane 3), t-PA (lane 4), plasminogen (lane 5), murine epidermal growth factor (lane 6), **ATF** (lane 7), active 54 kD u-PA (lane 8), DFP-inactivated 54 kD u-PA (lane 9). After preincubation for 15 min at room temperature, ^{125} I-labelled **ATF** (approximately 1 nM) was added, followed by incubation for 1 hour at 4° C. After incubation, chemical cross-linking was performed. . . by SDS-PAGE on a 6-16% gradient gel under non-reducing conditions and autoradiography. Lane 1 shows the cross-linked control with ^{125} I-**ATF** and no addition of u-PAR or competitors. Electrophoretic mobilities of molecular weight standard proteins are inducated (kD).

DRWD FIG. 3. Deglycosylation of cross-linked ^{125} I-**ATF**: u-PAR complexes from PMA-treated and nontreated U937a cells. PMA-treated (lanes 1 and 3) and nontreated (lanes 2 and 4) cells were acid-treated and lysed with 0.5% CHAPS. The lysates were incubated with ^{125} I-**ATF**, cross-linked with disuccinimidyl suberate, denatured under mildly reducing conditions, and then further incubated in the presence (lanes 3 and 4). . .

DRWD FIG. 5 shows chymotryptic fragments of u-PAR, analyzed by chemical cross-linking to ^{125} I-**ATF**. Preparation of samples and numbering of lanes are the same as in FIG. 4. The samples were 50-fold diluted and analyzed by chemical cross-linking to ^{125} I-**ATF**, followed by SDS-PAGE on a 6-16% gradient gel under reducing conditions, and autoradiography. The electrophoretic mobilities of molecular weight marker. . .

DRWD FIG. 6 shows deglycosylation of chymotryptic fragments, cross-linked to **ATF**. Samples of purified u-PAR were subjected to degradation with 8 ng/ml chymotrypsin (lanes 1 and 4) or 40 ng/ml chymotrypsin. . . incubated but received the same amount of phenylmethylsulfonylfluoride. The samples were 50-fold diluted, and subjected to chemical cross-linking to ^{125} I-**ATF**. The cross-linked samples were subjected to enzymatic deglycosylation with N-Glycanase (lanes 4-6) or treated in parallel without the addition of. . .

DRWD FIGS. 9A-9F. Caseinolytic plaque assay of **uPA** binding to LB6 cells transfected with p-uPAR-1 DNA. FIGS. 9A and C-F refer to clone LB6/p-uPAR-1 while plate B refers to clone LB6/RSVCAT. In plate A no **uPA** was added. Otherwise (FIGS. 9B-F) cells were subjected to a binding step with 0.2 nM human **uPA** for 1 hour at 37° C. The following competitors, present during the binding step, were used: none (FIGS. 9B, 9C); 100 nM **ATF** (FIG. 9D); 200 μM synthetic peptide human **uPA**[12-32(ala19)] (FIG. 9E); 100 μM synthetic peptide mouse **uPA**[13-33(ala20)] (FIG. 9F).

DRWD FIG. 10A. Binding of human ^{125} I-**ATF** to mouse LB6 cells transfected with RSVCAT (closed circles) and p-uPAR-1 DNA (closed

circles). Specific binding was calculated by subtracting the counts not competed by 100 nM unlabelled **ATF** (about 1000 cpm in this experiment).

DRWD FIG. 10B. Reducing SDS-polyacrylamide (12.5%) gel electrophoretic analysis of the ^{125} I-**ATF** cross-linked to LB6/p-uPAR-1 cells. Lane 1 has the molecular weight markers (see Methods); lane 2 represents the migration of the labelled **ATF** (3,000 cpm). Lanes 3 and 4 show the migration of duplicate LB6/p-uPAR-1 extracts cross-linked with ligand. Lanes 5 and 6 show the competition of the cross-linking of LB6/p-uPAR-1 cells to the ligand by unlabelled **ATF** (100 nM final concentration). The last lane to the right shows the cross-linking obtained (in a separate experiment) with the. . .

DRWD FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated **ATF**, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as described before. . . Similarly, conditioned medium was centrifuged at 100,000×g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated **ATF**, cross-linked with DSS, and analyzed by SDS-PAGE (part B of the Figure). Lanes a and b are duplicates from cells. . .

DRWD . . . addition of extra Triton X-114 and 0.1 M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM ^{125} I-labelled **ATF** was performed on parallel aliquots of aqueous (A) and detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to ^{125} I-**ATF**/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to ^{125} I-**ATF**. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to ^{125} I-**ATF**. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active **urokinase**. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal antibody to u-PA (anti-u-PA-ab). The bound. . .

DRWD FIGS. 29A-29B. Competition by unlabelled u-PA (.circle-solid.--.circle-solid.) or u-PA/PAI-1 complex (.smallcircle.--.smallcircle.) of the binding of ^{125} I-**ATF** to human U937 cells. [Competitor] is the concentration of free or PAI-1 complexed u-PA; for PAI-1/u-PA complex formation, a 50. . .

DRWD FIGS. 45A-45B. Inhibition of cellular **ATF** binding by antibodies raised against purified u-PAR. 5×10^5 U937a cells were preincubated with mouse antiserum raised against purified u-PAR (.circle-solid.--.circle-solid.). . . mouse antiserum raised against porcine mucins (.smallcircle.--.smallcircle.) for 1 hour at 4° C., followed by addition of 2.2 nM ^{125} I-**ATF** and incubation for another hour at the same temperature. The cells were then washed 3 times after which the cell-bound. . .

DETD . . . phosphate, 1.0 M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the ^{125} I-labelled amino terminal (**ATF**) fragment of **urokinase**, followed by

SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH_2 -terminal amino acid sequencing were dialyzed. .

.

DETD Protein labelling with ^{125} I. ^{125} I-labelling of **ATF** was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80.. . .

DETD Chemical cross-linking assay. Cross-linking of u-PAR in complex mixtures or purified fractions to ^{125} I-labelled **ATF** was performed as described for solubilized receptor (Nielsen et al., 1988), except that 2 mM disuccinimidylsuberate (DSS) was used for. . .

DETD . . . in cell lysates and detergent fractions, the receptor was selectively labelled before the degradation by chemical cross-linking to ^{125} I-labelled **ATF**.

DETD For desialylation, 70 µl lysate samples labelled by cross-linking to ^{125} I-**ATF**, were made up to 200 µl with 0.05 M sodium acetate, pH 5.0. 90 µl aliquots of the mixture received. . .

DETD Analysis for binding activity toward the **ATF** of **urokinase** was performed by chemical cross-linking to ^{125} I-labelled **ATF** followed by SDS-PAGE and autoradiography. **ATF**-binding activity co-eluted with silver-stainable protein. The conjugate formed between **ATF** and the purified protein migrated as a 70-75 kDa component during electrophoresis (FIG. 1B, lane 2). As demonstrated previously for partially purified u-PAR (Nielsen et al., 1988), the formed conjugate was indistinguishable from the cross-linked product formed with **ATF** on intact, PMA-stimulated U937 cells (not shown), as well as in non-purified detergent extracts from the same cells. Binding and cross-linking to ^{125} I-labelled **ATF** was specific and saturable. Thus, it could be competed for by an excess of unlabelled **ATF**, active u-PA or DFP-treated u-PA, while no competition was obtained with unrelated proteins such as, for example, bovine serum albumin,. . .

DETD . . . was performed with non-labelled components (FIG. 1C). In this experiment, DFP-treated u-PA was chosen as the u-PAR-specific ligand instead of **ATF**, since, because of the higher molecular weight, this ligand would lead to a conjugate clearly separable from the purified protein. . .

DETD . . . In these experiments, a selective labelling of u-PAR was performed before the deglycosylation reaction by chemical cross-linking to ^{125} I-labelled **amino terminal fragment** (**ATF**) of **urokinase** (Nielsen et al., 1988).

DETD . . . is seen (FIG. 3) that the cell lysates from which the receptor was purified gave rise to a 70-75 kDa u-PAR-**ATF** conjugate (lane 1) that could be deglycosylated to yield an approximately 50 kDa product (lane 3). **ATF** is known not to contain N-bound carbohydrate. Thus, as the change in apparent molecular weight was the same as that. . . protein above, this experiment provided independent evidence that the heavy glycosylation found is indeed a property of the only significant **ATF** binding component in the detergent lysates of these cells.

DETD When detergent lysates obtained from other cell lines were analyzed by chemical cross-linking to **ATF**, variations in the electrophoretic migration of the radiolabelled product were observed in certain cases. In these analyses, for comparison, individual. . .

DETD However, when samples representing the 4 patterns above were subjected to enzymatic deglycosylation after the cross-linking to ^{125} I-**ATF**, the molecular weight variation was abolished. The resulting conjugate band was sharp, and migrated as a 50 kDa component, irrespective. . .

DETD . . . detergent fractions from PMA-stimulated U937a cells led to an approximately 5 kDa reduction in the apparent molecular weight of the **ATF**-u-PAR conjugate. Thus, the glycosylation includes several sialic acid residues. The change in molecular weight, though undoubtedly present, appeared somewhat smaller. . .

DETD Samples to be analyzed by chemical cross-linking to ^{125} I-**ATF**

were 50-fold diluted in 0.1 M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition. . .

DETD Deglycosylation of Samples, Cross-Linked to ^{125} I-**ATF**

DETD . . . of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or **ATF** as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further. . .

DETD In parallel, the samples were analyzed in the chemical cross-linking assay, using ^{125} I-**ATF** as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which. . . to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD **ATF**. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare. . .

DETD . . . a 16 kD product, consistent with the expected size for the fragment with binding activity observed after cross-linking to ^{125} I-**ATF**. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system, suggesting that this fragment does. . .

DETD . . . essential medium (DMEM) supplemented with 10% foetal calf serum, 2 mM glutamine and 10 IU/ml of penicillin and streptomycin. Human **high molecular weight urokinase** and prourokinase were provided by Lepetit SpA (Nolli et al., 1989). The **amino terminal fragment** of human u-PA, **ATF**, was a gift from Abbott Laboratories. The synthetic peptides human u-PA[12-32(ala19)] and mouse u-PA[13-33(ala20)] have been described before (Appella et. . .

DETD . . . 9B), whereas those transfected with pRSVCAT DNA did not (see FIG. 9B). Specificity is shown by the ability of the **amino-terminal fragment** of u-PA (**ATF**), i.e. a truncated u-PA molecule maintaining the binding capacity but deprived of the catalytic activity (Stoppelli et al., 1985) (FIG.. . .

DETD . . . human u-PAR by mouse LB6 cells transfected with p-u-PAR-1 was further analysed by binding competition experiments using unlabelled and iodinated **ATF**. The molecular properties of the u-PAR expressed by the transfected cells were analysed by SDS-PACE and radiography of material from these cells cross-linked to iodinated **ATF**.

DETD Mouse LB6 cells were grown in DMEM as described in this Example. Iodination of **ATF** has been described previously by Stoppelli et al. (1985). The cross-linking reagent disuccinimidyl suberate was from Pierce Chemical Co.

DETD Binding of ^{125} I-**ATF**

DETD . . . bovine serum albumin, incubated in serum-free medium for 1 hour at 37° C., and then incubated with 47,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) at 37° C. for 60 minutes in the presence of different concentrations of unlabelled **ATF**. The experiment was carried out in duplicate. At the end of the incubation, the cells were washed with PBS-bovine serum. . . collected and counted (Stoppelli et al., 1985). Specific binding was calculated by subtracting the radioactivity not competed by 100 nM **ATF**.

DETD Cross-linking of ^{125} I-**ATF** to the u-PAR

DETD Cross-linking of LB6/p-u-PAR-1 cells with ^{125} I-**ATF** was carried out using disuccinimidyl suberate (DSS) as previously described (Picone et al., 1989). Duplicate dishes of 2.6×10^5 cells were washed with PBS-bovine serum albumin (1 mg/ml), incubated with 60,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) in serum-free DMEM supplemented with 25 mM Hepes, pH 7.4 for 60 minutes at 37° C., washed four. . . The cells were then lysed directly in Laemmli buffer containing 5% β-mercaptoethanol (Laemmli, 1970). In control samples, 100 nM unlabelled **ATF** was present during the binding step. The cell extract was analysed by SDS-polyacrylamide (12.5%) gel electrophoresis under reducing conditions (Laemmli,. . .

DETD Expression of p-u-PAR-1 DNA in LB6 cells is supported by quantitative

binding data with ^{125} I-**ATF**. FIG. 10A shows a binding-competition plot in which control LB6 cells (LBS/RSVCAT) do not bind ^{125} I-**ATF**, whereas LB6 cells transfected with p-u-PAR-1 DNA do. The binding is specifically competed by unlabelled **ATF**. Scatchard plot of the data gave a Ka of about 10^8 moles^{-1} and about 25,000 receptors/cell.

DETD . . . has the correct molecular properties, cross-linking studies were performed with the LB6/p-u-PAR-1 cells. Cells were incubated with human ^{125} I-labelled **ATF**, bound **ATF** cross-linked with disuccinimidyl suberate, the cells lysed and analysed by SDS-polyacrylamide gel electrophoresis. The results are shown in FIG. 10B.. . . with human GM637 cells (from which the cDNA clone is derived). This is the molecular weight expected for the intact **ATF**-u-PAR complex (Nielsen et al., 1988). Considering the possible cell-dependent difference in glycosylation, and the fact that PMA-treated cells possess a. . .

DETD . . . the human u-PAR gene in mouse LB6 cells by the following findings: p-u-PAR-1 DNA transfected LB6 cells bind labelled human **ATF** and unlabelled human u-PA as shown by direct binding assay (FIG. 10A) and the caseinolytic plaque assay (FIG. 9). The binding is specific as shown by the ability of human **ATF**, human synthetic peptide u-PA[12-32(ala19)], but not mouse synthetic peptide u-PA[13-33(ala20)] to compete for binding (FIGS. 9A-F and 10A). The **ATF**-u-PAR complex has the correct molecular weight (FIG. 10B).

DETD Production of a Soluble Receptor Protein Containing the Binding Site for **Urokinase**

DETD . . . u-PAR molecule that is partly recovered in the medium and partly retained in the cells. In fact, cross-linking to iodinated **ATF** shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example. . .

DETD . . . expected to be unable to attach to the cell surface, to be secreted in the medium, and to bind pro-u-PA, **ATF**, DFP-u-PA and active u-PA, in general the same molecules bound by the normal u-PA receptor. It should therefore be useful. . .

DETD . . . in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the **amino terminal fragment** (**ATF**) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). **ATF**, u-PAR and DFP-inhibited u-PA were radio-labelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0.01% (v/v) Tween 80 in the case of **ATF** and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

DETD . . . M NaCl (pH 7.5) and the cells were washed twice with buffer A. In some experiments exogenously added ^{125} I-labelled DFP-**uPA** (1 nM) were allowed to rebind to the unoccupied u-PAR by incubation for 2 hours at 4° C. in buffer. . .

DETD . . . PI-PLC was essentially non-degraded and consisted primarily of intact two-chain u-PA (Mr 50,000) along with a smaller amount of its **amino terminal fragment** (**ATF**, Mr 17,000). The receptor-binding domain of u-PA resides in both of these components (Appella et al., 1987). Accordingly, these two. . . u-PA (Mr 33,000), devoid of the receptor-binding domain, was eliminated by the washing procedures. These data indicate that u-PA and **ATF** were released from the cell surface by PI-PLC, while they were specifically associated to u-PAR.

DETD . . . soluble protein (Mr 60,000) that still expressed high affinity towards ^{125} I-labelled DFP-u-PA (FIG. 14C) as well as ^{125} I-labelled **ATF** (data not shown). Furthermore, by SDS-PAGE and immunoblotting, a protein with similar Mr was detected in the serum-free medium after. . .

DETD . . . detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to, ^{125} I-labelled **ATF** (FIG. 15A), thus

demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the **ATF**-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this. . .

DETD . . . detergents in the polyacrylamide gel (data not shown). This experiment shows that the PI-PLC induced change in phase-partitioning of the **ATF** binding activity is totally accounted for by an identical change in the hydrophobicity of the u-PAR protein itself.

DETD The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. ^{125} I-labelled aminoterminal fragment (**ATF**) of the **urokinase** were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of PMA treatment both an increase in the strength. . .

DETD . . . of u-PAR protein was studied by the cross linking assay as described. FIG. 20 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of dibuturyl cAMP treatment both an increase in the. . .

DETD . . . et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the proenzyme of **urokinase**-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum,. . . human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates **urokinase**-type plasminogen activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung,. . . IgG antibody to human u-PA (clone 2 (10 μg/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human **urokinase**-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic. . .

DETD . . . against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble **urokinase**.

DETD . . . Peeters, Ed.), 33, 623-626, 1985, and was a kind gift from E. Sarubbi and A. Soffientini. Two-chain u-PA and u-PA **amino-terminal fragment** (**ATF**) purification (Stoppelli et al., 1985) and DFP-treated u-PA preparation (Andreasen et al., 1986) have previously been described. Human plasmin (4. . .

DETD Iodinations. 1 μg portions of protein (**ATF**, u-PA or pro-u-PA) in 30 mM sodium phosphate buffer (pH 7.4) were iodinated with 1 mCi of Na^{125} I (Amersham. . .

DETD . . . (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for **ATF** and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4° C. After binding, the cells. . .

DETD Effect of u-PA and u-PA/PAI-1 Complex on Binding of ^{125} I-**ATF** to the u-PA Receptor

DETD In order to study the interaction between PAI-1 and receptor-bound u-PA, it was first tested whether purified PAI-1 competes with **ATF** for binding to the receptor on U937 cells, and it was found that it does not, event at a 1000:1 excess (data not shown). Then, the ability of unlabelled u-PA and preformed u-PA/PAI-1 complex to compete with ^{125} I-**ATF** for receptor binding was compared. FIGS. 29A-B show the dependence of the inhibition of ^{125} I-**ATF** binding to U937 cells on the concentration of unlabelled u-PA or u-PA/PAI-1 complex. Since PAI-1 forms stoichiometric covalent complexes with. . . presented in FIGS. 29A-B indicate that complexing of u-PA by PAI-1 does not

dramatically alter its ability to compete with **ATF** for receptor binding. The slight difference in the shape of the competition curves, suggesting that u-PA is a 2-3 fold. . .

DETD . . . migration of the u-PA/PAI-1 complex. This band represents receptor-bound u-PA/PAI-1 complex as it is competed for by unlabelled 85 nM **ATF** or u-PA.

DETD . . . receptor was further investigated. u-PA binds the receptor through its amino-terminal extremity, and the binding is competed equally well by **ATF** or u-PA (Stoppelli et al., 1985). Accordingly, it was found that the binding of the u-PA/PAI-1 complex can be competed to the same extent by **ATF** and u-PA, with 50% competition reached around 1-2 nM (data not shown). Thus, even when complexed to its inhibitor, u-PA. . .

DETD . . . (step 1). In all cases, more than 90% of the binding occurring during step 1 was inhibited by u-PA or **ATF** while no inhibition was obtained with low molecular weight u-PA, demonstrating the specificity of the interaction (data not shown). In. . .

DETD Four different iodinated ligands were tested: two-chain u-PA (u-PA), DFP-inactivated u-PA (DFP-u-PA), the **amino-terminal fragment** of u-PA (**ATF**) and the preformed u-PA:PAI-1 complex. The amount of receptor-bound ligand (cell-associated, acid-extracted radioactivity), of cell-trapped ligand (cell-associated, acid-resistant radioactivity) and. . . below). FIG. 33 shows the fate of the ligand during step 2 incubation at 37° C. In the case of **ATF** and DFP-u-PA, the receptor-bound fraction decreases slowly in agreement with previous data (Stoppelli et al., 1985); for u-PA, the decrease. . . little complex is found still to be surface-bound. The non-degraded, internalized ligand constitutes a small fraction in the case of **ATF**, but is clearly higher in all other cases. In particular in the case of the u-PA:PAI-1 complex, it increases rapidly. . . of the total radioactivity around 30 minutes, and decreasing thereafter. While very little ligand is degraded in the case of **ATF** and DFP-u-PA, a larger fraction is degraded in the case of u-PA (20% after 3 hours) and much more in. . . time course suggests a precursor-product relationship between the cell-trapped and the degraded ligand. Possibly, therefore, the u-PA:PAI-1 complex, but not **ATF** and DFP-u-PA, is internalized and then degraded. In the experiment shown in FIGS. 33A-D, u-PA might represent an intermediate case. . .

DETD The results unequivocally show that while **ATF**, DFP-treated u-PA and free active u-PA (in particular when excess low molecular weight u-PA is present to titrate endogenous inhibitors). . .

DETD Previous data have shown the absence of internalization of receptor-bound **ATF**, u-PA and pro-u-PA (Vassali et al., 1985; Stoppelli et al., 1985; Bajpai and Baker, 1985a; Stoppelli et al., 1986). These. . .

DETD . . . Plasminogen isoform 2 was used in all experiments described here. u-PA (M_r 55,000) was obtained either by plasmin activation of pro-**uPA** (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide. . . al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-**urokinase** (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The. . .

DETD . . . were then removed at various time points. The presence of u-PAR in these supernatants was demonstrated by cross-linking to ^{125}I-**ATF** using DSS as described in Example 1. The effect of this soluble form of u-PAR on u-PA enzymatic activity was. . .

DETD Supernatants from PI-PLC-treated PMA-stimulated U937 cells contain a soluble form of u-PAR, as determined by DSS crosslinking to ^{125}I-**ATF**. When these supernatants were incubated with u-PA, there was a concentration-dependent decrease in u-PA activity (FIG. 41) which was much. . . observed with the control supernatants, which is due to the

small amounts of u-PAR observed in the sample by ^{125} I-**ATF** cross-linking.

DETD Mice of the BALB/c strain were immunized with u-PAR purified on a diisopropylfluoride **urokinase**-type plasminogen activator (DFP-u-PA) ligand affinity column. The mice were given three intraperitoneal injections with 5 μg of u-PAR with 3. . .

DETD 6) Blocking buffer: 25% fetal calf serum in PBS (25% FCS/PBS) or 1% skimmed milk **powder** (SMP) in PBS.

DETD . . . piece was lyophilized and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide **powder** was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit.. . .

DETD Assay for Inhibition of Cellular **ATF** Binding--U937 cells were washed and acid-treated, as described (Nielsen et al., 1988). The cells were resuspended in 100 μl of. . . The samples were incubated for 1 hour at 4° C. with gentle stirring. After the incubation, 100 μl of ^{125} I-**ATF** was added and incubation was continued for another hour. In the 300-μl reaction volume, the final concentration of ^{125} I-**ATF** was 2.2 nM, and the final dilutions of anti-u-PAR serum/control serum ranged from 1:300 to 1:153,600. The cells were then. . .

DETD . . . was used in a competition experiment in which U937 cells were preincubated with the antiserum followed by addition of ^{125} I-**ATF**. As shown in FIGS. 45A-B, the anti-u-PAR serum was able to completely inhibit the specific binding of ^{125} I-**ATF** to the cells. 50% inhibition was obtained at a 1:2400 dilution. Under the same conditions, a control serum showed only. . .

DETD . . . serum (final IgG concentration 90 μg/ml during preincubation). This treatment completely hindered the subsequent formation of cross-linked conjugates with ^{125} I-**ATF**. The IgG from the pre-immune serum had no effect on the cross-linking assay at the same concentration.

DETD . . . at 37° C. This treatment led to an approx. 50% delipidation of u-PAR as judged by the shift of the **ATF** cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see Example 1).

DETD . . . S, Kristensen P, Gr.o slashed.ndahl-Hansen J, Skriver L, Dan.o slashed. K (1986) Plasminogen activator inhibitor from human fibrosarcoma cells binds **urokinase**-type plasminogen activator, but not its proenzyme. J Biol Chem 261: 7644-7651

DETD . . . Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani G, Blasi F (1987) The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

DETD Bajpai A, Baker J B (1985) Cryptic **urokinase** binding sites on human foreskin fibroblasts. Biochem Biophys Res Commun 133: 475-482

DETD Blasi F (1988) Surface receptors for **urokinase plasminogen activator**. Fibrinolysis 2: 73-84

DETD Blasi F, Stoppelli M P, Cubellis M V (1986) The receptor for **urokinase-plasminogen activator**. J Cell Biochem 32: 179-186

DETD Blasi F, Vassalli J-D, Dan.o slashed. K (1987) **Urokinase**-type plasminogen activator: proenzyme, receptor, and inhibitors. J Cell Biol 104: 801-804

DETD Boyd D, Florent G, Kim P, Brattain M (1988a) Determination of the levels of **urokinase** and its receptor in human colon carcinoma cell lines. Cancer Res 48: 3112-3116

DETD Boyd D, Florent G, Murano G, Brattain M (1988b) Modulation of the **urokinase** receptor in human colon cell lines by N,N-dimethylformamide. Biochim Biophys Acta 947: 96-100

DETD Collen D, Zamarron C, Lijnen H R, Hoylaerts M (1986) Activation of plasminogen by pro-**urokinase**. II. Kinetics. J Biol Chem 261: 1259-1266

DETD Cubellis M V, Nolli M L, Cassani G, Blasi F (1986) Binding of single-chain pro-**urokinase** to the **urokinase** receptor of human U937 cells. J Biol Chem 261: 15819-15822

DETD Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast **urokinase** proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

DETD Ellis V, Scully M F, Kakkar V V (1987) Plasminogen activation by single-chain **urokinase** in functional isolation. J Biol Chem 262: 14998-15003

DETD Ellis V, Scully M F, Kakkar V V (1988) Role of human U937 monocytes in controlling single-chain **urokinase**-initiated plasminogen activation. Fibrinolysis 2: supp. 1, 112

DETD Ellis V, Scully M F, Kakkar V V (1989) Plasminogen aktivation initiated by single-chain **urokinase**-type plasminogen activator. J Biol Chem 264, 2185-88

DETD Estreicher A, Wohlwend A, Belin D, Schleuning W-D, Vassalli J-D (1989) Characterization of the cellular binding site for the **urokinase**-type plasminogen activator. J Biol Chem 264: 1180-1189

DETD . . . Munkholm-Larsen P, Bach F, Nielsen L S, Dombernowsky P, Dan.o slashed. K (1988) Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. J Lab Clin Med 111: 42-51

DETD Gr.o slashed.ndahl-Hansen J, Lund L R, Ralfki.ae butted.r E, Ottevanger V, Dan.o slashed. K (1988) **Urokinase**- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. The Journal of Investigative Dermatology 90: 790-795

DETD . . . S, Kelley P, Suddith R L, Greenlee R (1984) Effective and fibrin-specific clot lysis by a zymogen precursor form of **urokinase** (pro-**urokinase**). A study In Vitro and in two animal species. J Clin Invest 73: 1731-1739

DETD Hearing V J, Law L W, Corti A, Appella E, Blasi F (1988) Modulation of metastatic potential by cell surface **urokinase** of murine melanoma cells. Cancer Res 48: 1270-1278

DETD Hebert C A, Baker J B (1988) Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: Colocalization of cell surface **urokinase** with vinculin. J Cell Biol 106: 1241-1247

DETD Janicke F, Schmitt M, Hafter A, Hollrieder A, Babic R, Ulm K, Gossner W, Graeff H (1990) **Urokinase**-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1-10

DETD Janicke F, Schmitt M, Ulm K, Gossner W, Graeff H (1989) **Urokinase**-type plasminogen activator antigen and early relapse in breast cancer. The Lancet, 1049

DETD Kasai S, Arimura H. Nishida M, Suyama T (1985) Proteolytic cleavage of single-chain pro-**urokinase** induces conformational change which follows activation of the zymogen and reduction of its high affinity for fibrin. J Biol Chem. . .

DETD Kielberg V, Andreasen P A, Gr.o slashed.ndahl-Hansen J, Nielsen L S, Skriver L, Dan.o slashed. K (1985) Proenzyme to **urokinase**-type plasminogen activator in the mouse in vivo. FEBS Lett 182: 441-445

DETD Lijnen H R, Zamarron C, Blaber M, Winkler M E, Collen D (1986) Activation of plasminogen by pro-**urokinase**. I. Mechanism. J Biol Chem 261: 1253-1258

DETD Miles L A, Plow E F (1987) Receptor mediated binding of the fibrinolytic components, plasminogen and **urokinase**, to peripheral blood cells. Thromb Haemostas 58: 936-942

DETD Needham G K, Sherbet G V, Farndon J R, Harris A L (1987) Binding of **urokinase** to specific receptor sites on human breast cancer membranes. Eur J Cancer 55: 13-16

DETD Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain **urokinase**-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689

DETD . . . S, Kellerman G M, Behrendt N, Picone R, Dan.o slashed. K, Blasi

F (1988) A 55,000-60,000 M_r receptor protein for **urokinase**-type plasminogen activator, J Biol Chem 263: 2358-2363

DETD . . . A, Robbiati F, Soffientini A, Blasi F, Parenti F, Cassani G (1989) Production and characterization of human recombinant single chain **urokinase**-type plasminogen activator from mouse cells. Fibrinolysis 3: 101-106

DETD Pannell R, Gurewich V (1987) Activation of plasminogen by single-chain **urokinase** or by two-chain **urokinase**--a demonstration that single-chain **urokinase** has a low catalytic activity (pro-**urokinase**). Blood 69: 22-26

DETD Petersen L C, Lund L R, Nielsen L S, Dan.o slashed. K, Skriver L (1988) One-chain **urokinase**-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J Biol Chem 263: 11189-11195

DETD . . . N, Mastronicola M R, Cubellis M V, Stoppelli M P, Pedersen S, Dan.o slashed. K, Blasi F (1989) Regulation of **urokinase** receptors in monocyte-like U937 cells by phorbol ester PMA. J Cell Biol 108: 693-702

DETD . . . F, Freaney D E, Plescia J, Miles L A (1986) The plasminogen system and cell surfaces: evidence for plasminogen and **urokinase** receptors on the same cell type. J Cell Biol 103: 2411-2420

DETD Pollanen J, Hedman K, Nielsen L S, Dan.o slashed. K, Vaheri A (1988) Ultra-structural localization of plasma membrane-associated **urokinase**-type plasminogen activator at focal contacts. J Cell Biol 106: 87-95

DETD Pollanen J, Saksela O, Salonen E-M, Andreasen P, Nielsen L S, Dan.o slashed. K, Vaheri A (1987) Distinct localizations of **urokinase**-type plasminogen activator and its type-1 inhibitor under cultured human fibroblasts and sarcoma cells. J Cell Biol 104: 1085-1096

DETD . . . L, Larsson L-I, Kielberg V, Nielsen L S, Andresen P B, Kristensen P, Dan.o slashed. K (1984) Immunocytochemical localization of **urokinase**-type plasminogen activator in Lewis lung carcinoma. J Cell Biol 99: 753-758

DETD Stephens R W, Alitalo R, Tapiovaara H, Vaheri A (1988) Production of an active **urokinase** by leukemia cell lines: a novel distinction from cell lines of solid tumors. Leukemia Res 12: 419-422

DETD Stephens R W, Fordham C J, Doe W F (1987) Proenzyme to **urokinase**-type plasminogen activator in human colon cancer: in vitro inhibition by monocyte minactivin after proteolytic activation. Eur J Cancer Clin Oncol. . .

DETD Stoppelli M P, Corti A, Soffientini A, Cassani G, Blasi F, Assoian R K (1985) Differentiation-enhanced binding of the **amino-terminal fragment** of human **urokinase plasminogen activator** to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA 82: 4939-4943

DETD . . . Tacchetti C, Cubellis M V, Corti A, Hearing V J, Cassani G, Appella E, Blasi F (1986) Autocrine saturation of pro-**urokinase** receptors on human A431 cells. Cell 45: 675-684

DETD Stump D C, Lijnen H R, Collen D (1986a) Purification and characterization of single-chain **urokinase**-type plasminogen activator from human cell cultures. J Biol Chem 261: 1274-1278

DETD Stump D C, Thienpont M, Collen D (1986b) **Urokinase**-related proteins in human urine. J Biol Chem 261: 1267-1273

DETD Thorsen S, Glas-Greenwalt P, Astrup T (1972) Difference in the binding to fibrin of **urokinase** and tissue plasminogen activator. Thrombos Diathes Haemorrh 28: 65-74

DETD Urano T, de Serrano VS, Gaffney PJ, Castellino FJ (1988) The activation of human (Glu^1) plasminogen by human single-chain **urokinase**. Arch Biochem Biophys 264: 222-230

DETD . . . J-D, Baccino D, Belin D (1985) A cellular binding site for the M_r 55,000 form of the human plasminogen activator, **urokinase**. J Cell Biol 100: 86-92

DETD Wun T-C. Ossowski L, Reich E (1982) A proenzyme form of human **urokinase**. J Biol Chem 157: 7262-7268

CLM What is claimed is:

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PAR) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting. . .

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .

5. A method according to claim 2 wherein the modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA).

8. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .

24. A method of inhibiting the binding of **urokinase** type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase plasminogen-activator** receptor (u-PAR) which comprises contacting a plasminogen-converting, receptor-binding form of u-PA or pro-u-PA, or a receptor (u-PAR) for said form. . .

26. A method of inhibiting the conversion of plasminogen to plasmin which comprises inhibiting the binding of a plasminogen-converting, **urokinase-plasminogen-activator** receptor by the method of claim 24, and hence inhibiting the consequent conversion of plasminogen to plasmin.

28. The method of claim 27 in which the substance is capable of inhibiting binding of u-PA or pro-**UPA** to u-PAR in a supernatant of HT-1080 cells.

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     FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1          9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2           231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3           106 S L2 AND AD<MAR 01 2001
L4             8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)
L5            25 S L3 AND POWDER
L6            19 S L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L7             1 S L4 AND L6

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L8             1 L7 AND (POWDER)

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          35868 LECITHIN
          38206 METHYLCELLULOSE
          58838 MANNITOL
         101549 LACTOSE
L9             1 L8 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
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L9 ANSWER 1 OF 1 USPATFULL on STN
TI **Urokinase**-type plasminogen activator receptor
AI US 1995-442108 19950516 (8) <--
AB Activation of plasminogen to plasmin is inhibited by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator to a **urokinase**-type plasminogen activator receptor in a mammal, thereby preventing the **urokinase**-type plasminogen activator from converting plasminogen into plasmin. DNA fragments which encode for soluble, active fragments of the **urokinase**-type plasminogen activator receptor are provided.
SUMM . . . the method comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator (in the following termed u-PA) to a u-PA receptor in the mammal and thereby preventing the u-PA from. . .
SUMM According to the literature, **urokinase**-type plasminogen activator (u-PA) has been found in all mammalian species so far investigated. Several findings relate u-PA to tissue degradation. . .
SUMM . . . in the primary structure is remote from the catalytic site. The receptor binding domain is located in the 15 kD **amino-terminal fragment** (**ATF**, residues 1-135) of the u-PA molecule, more precisely within the cysteine-rich region termed the growth factor region as this region. . .
SUMM . . . al., 1985, Vassalli et al., 1985, Nielsen et al., 1988). Fragments of u-PA containing only the receptor binding domain, e.g. **ATF**, ensure specificity of the binding to the receptor, since other molecules that might bind u-PA (protease nexin and the specific. . .
SUMM . . . al., 1989, binding of the added single-chain u-PA to the receptor was prevented by preincubation of the cells with the **amino-terminal fragment** of u-PA. These experiments do not, therefore, as do the following examples, demonstrate displacement of endogenously produced u-PA, a prerequisite. . .
SUMM Human tumor cells are very commonly found to secrete plasminogen activator of the **urokinase** type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen. . .
SUMM . . . streptavidin-fluorescin isothiocyanate. The method is very sensitive, and its specificity can readily be tested by competition experiments (e.g. with the **amino-terminal fragment** of u-PA (**ATF**), t-PA, EGF, etc.).
SUMM While the present specification and claims relate predominantly to the **urokinase** type plasminogen activator (u-PA), it is obvious that the same approach can and should be used for tissue-type plasminogen activator. . .
SUMM . . . that the u-PA contains the u-PAR binding site. The receptor binding form of u-PA can thus be pro-u-PA, u-PA, an **amino-terminal fragment** of u-PA, a u-PA that is irreversibly inhibited by e.g. diisopropyl fluorophosphate (DFP), p-nitrophenyl-p'-guanidinobenzoate (NPGB), or any other inhibitor or. . .
SUMM The enzyme **urokinase**-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg^{560}, plasminogen is activated to the. . .
SUMM Another useful modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA) (cf. Stoppelli et al., 1985).
SUMM . . . mg such as about 150 mg for an average adult person. The same considerations apply with respect to NPGB-u-PA, the **amino-terminal fragment** of u-PA, and pro-u-PA that is modified so that it cannot be cleaved by plasmin. Evidently, the higher the affinity. . .
SUMM . . . of substrates which are useful in the present method as substrates for the enzymes mentioned above are H_2 O_2,

p-nitrophenylphosphate, **lactose**, urea, β-D-glucose, CO_2, RNA, starch, or malate. The substrate may be combined with, e.g. a chromophore which is either a. . .

DRWD . . . of 100 nM: bovine serum albumin (lane 3), t-PA (lane 4), plasminogen (lane 5), murine epidermal growth factor (lane 6), **ATF** (lane 7), active 54 kD u-PA (lane 8), DFP-inactivated 54 kD u-PA (lane 9). After preincubation for 15 min at room temperature, ^{125} I-labelled **ATF** (approximately 1 nM) was added, followed by incubation for 1 hour at 4° C. After incubation, chemical cross-linking was performed. . . by SDS-PAGE on a 6-16% gradient gel under non-reducing conditions and autoradiography. Lane 1 shows the cross-linked control with ^{125} I-**ATF** and no addition of u-PAR or competitors. Electrophoretic mobilities of molecular weight standard proteins are inducated (kD).

DRWD FIG. 3. Deglycosylation of cross-linked ^{125} I-**ATF**: u-PAR complexes from PMA-treated and nontreated U937a cells. PMA-treated (lanes 1 and 3) and nontreated (lanes 2 and 4) cells were acid-treated and lysed with 0.5% CHAPS. The lysates were incubated with ^{125} I-**ATF**, cross-linked with disuccinimidyl suberate, denatured under mildly reducing conditions, and then further incubated in the presence (lanes 3 and 4). . .

DRWD FIG. 5 shows chymotryptic fragments of u-PAR, analyzed by chemical cross-linking to ^{125} I-**ATF**. Preparation of samples and numbering of lanes are the same as in FIG. 4. The samples were 50-fold diluted and analyzed by chemical cross-linking to ^{125} I-**ATF**, followed by SDS-PAGE on a 6-16% gradient gel under reducing conditions, and autoradiography. The electrophoretic mobilities of molecular weight marker. . .

DRWD FIG. 6 shows deglycosylation of chymotryptic fragments, cross-linked to **ATF**. Samples of purified u-PAR were subjected to degradation with 8 ng/ml chymotrypsin (lanes 1 and 4) or 40 ng/ml chymotrypsin. . . incubated but received the same amount of phenylmethylsulfonylfluoride. The samples were 50-fold diluted, and subjected to chemical cross-linking to ^{125} I-**ATF**. The cross-linked samples were subjected to enzymatic deglycosylation with N-Glycanase (lanes 4-6) or treated in parallel without the addition of. . .

DRWD FIGS. 9A-9F. Caseinolytic plaque assay of **uPA** binding to LB6 cells transfected with p-uPAR-1 DNA. FIGS. 9A and C-F refer to clone LB6/p-uPAR-1 while plate B refers to clone LB6/RSVCAT. In plate A no **uPA** was added. Otherwise (FIGS. 9B-F) cells were subjected to a binding step with 0.2 nM human **uPA** for 1 hour at 37° C. The following competitors, present during the binding step, were used: none (FIGS. 9B, 9C); 100 nM **ATF** (FIG. 9D); 200 μM synthetic peptide human **uPA**[12-32(ala19)] (FIG. 9E); 100 μM synthetic peptide mouse **uPA**[13-33(ala20)] (FIG. 9F).

DRWD FIG. 10A. Binding of human ^{125} I-**ATF** to mouse LB6 cells transfected with RSVCAT (closed circles) and p-uPAR-1 DNA (closed circles). Specific binding was calculated by subtracting the counts not competed by 100 nM unlabelled **ATF** (about 1000 cpm in this experiment).

DRWD FIG. 10B. Reducing SDS-polyacrylamide (12.5%) gel electrophoretic analysis of the ^{125} I-**ATF** cross-linked to LB6/p-uPAR-1 cells. Lane 1 has the molecular weight markers (see Methods); lane 2 represents the migration of the labelled **ATF** (3,000 cpm). Lanes 3 and 4 show the migration of duplicate LB6/p-uPAR-1 extracts cross-linked with ligand. Lanes 5 and 6 show the competition of the cross-linking of LB6/p-uPAR-1 cells to the ligand by unlabelled **ATF** (100 nM final concentration). The last lane to the right shows the cross-linking obtained (in a separate experiment) with the. . .

DRWD FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated **ATF**, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as

described before. . . Similarly, conditioned medium was centrifuged at 100,000×g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated **ATF**, cross-linked with DSS, and analyzed by SDS-PAGE (part B of the Figure). Lanes a and b are duplicates from cells. . .

DRWD . . . addition of extra Triton X-114 and 0.1 M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM ^{125} I-labelled **ATF** was performed on parallel aliquots of aqueous (A) and detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to ^{125} I-**ATF**/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to ^{125} I-**ATF**. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to ^{125} I-**ATF**. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active **urokinase**. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal antibody to u-PA (anti-u-PA-ab). The bound. . .

DRWD FIGS. 29A-29B. Competition by unlabelled u-PA (.circle-solid.--.circle-solid.) or u-PA/PAI-1 complex (.smallcircle.--.smallcircle.) of the binding of ^{125} I-**ATF** to human U937 cells. [Competitor] is the concentration of free or PAI-1 complexed u-PA; for PAI-1/u-PA complex formation, a 50. . .

DRWD FIGS. 45A-45B. Inhibition of cellular **ATF** binding by antibodies raised against purified u-PAR. 5×10^5 U937a cells were preincubated with mouse antiserum raised against purified u-PAR (.circle-solid.--.circle-solid.). . . mouse antiserum raised against porcine mucins (.smallcircle.--.smallcircle.) for 1 hour at 4° C., followed by addition of 2.2 nM ^{125} I-**ATF** and incubation for another hour at the same temperature. The cells were then washed 3 times after which the cell-bound. . .

DETD . . . phosphate, 1.0 M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the ^{125} I-labelled amino terminal (**ATF**) fragment of **urokinase**, followed by SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH_2 -terminal amino acid sequencing were dialyzed. .

.

DETD Protein labelling with ^{125} I. ^{125} I-labelling of **ATF** was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80.. . .

DETD Chemical cross-linking assay. Cross-linking of u-PAR in complex mixtures or purified fractions to ^{125} I-labelled **ATF** was performed as described for solubilized receptor (Nielsen et al., 1988), except that 2 mM disuccinimidylsuberate (DSS) was used for. . .

DETD . . . in cell lysates and detergent fractions, the receptor was selectively labelled before the degradation by chemical cross-linking to ^{125} I-labelled **ATF**.

DETD For desialylation, 70 µl lysate samples labelled by cross-linking to ^{125} I-**ATF**, were made up to 200 µl with 0.05 M sodium acetate,

pH 5.0. 90 µl aliquots of the mixture received. . .

DETD Analysis for binding activity toward the **ATF** of **urokinase** was performed by chemical cross-linking to ^{125} I-labelled **ATF** followed by SDS-PAGE and autoradiography. **ATF**-binding activity co-eluted with silver-stainable protein. The conjugate formed between **ATF** and the purified protein migrated as a 70-75 kDa component during electrophoresis (FIG. 1B, lane 2). As demonstrated previously for partially purified u-PAR (Nielsen et al., 1988), the formed conjugate was indistinguishable from the cross-linked product formed with **ATF** on intact, PMA-stimulated U937 cells (not shown), as well as in non-purified detergent extracts from the same cells. Binding and cross-linking to ^{125} I-labelled **ATF** was specific and saturable. Thus, it could be competed for by an excess of unlabelled **ATF**, active u-PA or DFP-treated u-PA, while no competition was obtained with unrelated proteins such as, for example, bovine serum albumin,. . .

DETD . . . was performed with non-labelled components (FIG. 1C). In this experiment, DFP-treated u-PA was chosen as the u-PAR-specific ligand instead of **ATF**, since, because of the higher molecular weight, this ligand would lead to a conjugate clearly separable from the purified protein. . .

DETD . . . In these experiments, a selective labelling of u-PAR was performed before the deglycosylation reaction by chemical cross-linking to ^{125} I-labelled **amino terminal fragment** (**ATF**) of **urokinase** (Nielsen et al., 1988).

DETD . . . is seen (FIG. 3) that the cell lysates from which the receptor was purified gave rise to a 70-75 kDa u-PAR-**ATF** conjugate (lane 1) that could be deglycosylated to yield an approximately 50 kDa product (lane 3). **ATF** is known not to contain N-bound carbohydrate. Thus, as the change in apparent molecular weight was the same as that. . . protein above, this experiment provided independent evidence that the heavy glycosylation found is indeed a property of the only significant **ATF** binding component in the detergent lysates of these cells.

DETD When detergent lysates obtained from other cell lines were analyzed by chemical cross-linking to **ATF**, variations in the electrophoretic migration of the radiolabelled product were observed in certain cases. In these analyses, for comparison, individual. . .

DETD However, when samples representing the 4 patterns above were subjected to enzymatic deglycosylation after the cross-linking to ^{125} I-**ATF**, the molecular weight variation was abolished. The resulting conjugate band was sharp, and migrated as a 50 kDa component, irrespective. . .

DETD . . . detergent fractions from PMA-stimulated U937a cells led to an approximately 5 kDa reduction in the apparent molecular weight of the **ATF**-u-PAR conjugate. Thus, the glycosylation includes several sialic acid residues. The change in molecular weight, though undoubtedly present, appeared somewhat smaller. . .

DETD Samples to be analyzed by chemical cross-linking to ^{125} I-**ATF** were 50-fold diluted in 0.1 M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition. . .

DETD Deglycosylation of Samples, Cross-Linked to ^{125} I-**ATF**

DETD . . . of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or **ATF** as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further. . .

DETD In parallel, the samples were analyzed in the chemical cross-linking assay, using ^{125} I-**ATF** as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which. . . to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD **ATF**. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare. . .

DETD . . . a 16 kD product, consistent with the expected size for the

fragment with binding activity observed after cross-linking to ^{125} I-**ATF**. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system, suggesting that this fragment does. . .

DETD . . . essential medium (DMEM) supplemented with 10% foetal calf serum, 2 mM glutamine and 10 IU/ml of penicillin and streptomycin. Human **high molecular weight urokinase** and prourokinase were provided by Lepetit SpA (Nolli et al., 1989). The **amino terminal fragment** of human u-PA, **ATF**, was a gift from Abbott Laboratories. The synthetic peptides human u-PA[12-32(ala19)] and mouse u-PA[13-33(ala20)] have been described before (Appella et. . .

DETD . . . 9B), whereas those transfected with pRSVCAT DNA did not (see FIG. 9B). Specificity is shown by the ability of the **amino-terminal fragment** of u-PA (**ATF**), i.e. a truncated u-PA molecule maintaining the binding capacity but deprived of the catalytic activity (Stoppelli et al., 1985) (FIG.. . .

DETD . . . human u-PAR by mouse LB6 cells transfected with p-u-PAR-1 was further analysed by binding competition experiments using unlabelled and iodinated **ATF**. The molecular properties of the u-PAR expressed by the transfected cells were analysed by SDS-PACE and radiography of material from these cells cross-linked to iodinated **ATF**.

DETD Mouse LB6 cells were grown in DMEM as described in this Example. Iodination of **ATF** has been described previously by Stoppelli et al. (1985). The cross-linking reagent disuccinimidyl suberate was from Pierce Chemical Co.

DETD Binding of ^{125} I-**ATF**

DETD . . . bovine serum albumin, incubated in serum-free medium for 1 hour at 37° C., and then incubated with 47,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) at 37° C. for 60 minutes in the presence of different concentrations of unlabelled **ATF**. The experiment was carried out in duplicate. At the end of the incubation, the cells were washed with PBS-bovine serum. . . collected and counted (Stoppelli et al., 1985). Specific binding was calculated by subtracting the radioactivity not competed by 100 nM **ATF**.

DETD Cross-linking of ^{125} I-**ATF** to the u-PAR

DETD Cross-linking of LB6/p-u-PAR-1 cells with ^{125} I-**ATF** was carried out using disuccinimidyl suberate (DSS) as previously described (Picone et al., 1989). Duplicate dishes of 2.6×10^5 cells were washed with PBS-bovine serum albumin (1 mg/ml), incubated with 60,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) in serum-free DMEM supplemented with 25 mM Hepes, pH 7.4 for 60 minutes at 37° C., washed four. . . The cells were then lysed directly in Laemmli buffer containing 5% β-mercaptoethanol (Laemmli, 1970). In control samples, 100 nM unlabelled **ATF** was present during the binding step. The cell extract was analysed by SDS-polyacrylamide (12.5%) gel electrophoresis under reducing conditions (Laemmli,. . .

DETD Expression of p-u-PAR-1 DNA in LB6 cells is supported by quantitative binding data with ^{125} I-**ATF**. FIG. 10A shows a binding-competition plot in which control LB6 cells (LBS/RSVCAT) do not bind ^{125} I-**ATF**, whereas LB6 cells transfected with p-u-PAR-1 DNA do. The binding is specifically competed by unlabelled **ATF**. Scatchard plot of the data gave a Ka of about 10^8 moles^{-1} and about 25,000 receptors/cell.

DETD . . . has the correct molecular properties, cross-linking studies were performed with the LB6/p-u-PAR-1 cells. Cells were incubated with human ^{125} I-labelled **ATF**, bound **ATF** cross-linked with disuccinimidyl suberate, the cells lysed and analysed by SDS-polyacrylamide gel electrophoresis. The results are shown in FIG. 10B.. . . with human GM637 cells (from which the cDNA clone is derived). This is the molecular weight expected for the intact **ATF**-u-PAR complex (Nielsen et al., 1988). Considering the possible cell-dependent difference in glycosylation, and the fact that

PMA-treated cells possess a. . .

DETD . . . the human u-PAR gene in mouse LB6 cells by the following findings: p-u-PAR-1 DNA transfected LB6 cells bind labelled human **ATF** and unlabelled human u-PA as shown by direct binding assay (FIG. 10A) and the caseinolytic plaque assay (FIG. 9). The binding is specific as shown by the ability of human **ATF**, human synthetic peptide u-PA[12-32(ala19)], but not mouse synthetic peptide u-PA[13-33(ala20)] to compete for binding (FIGS. 9A-F and 10A). The **ATF**-u-PAR complex has the correct molecular weight (FIG. 10B).

DETD Production of a Soluble Receptor Protein Containing the Binding Site for **Urokinase**

DETD . . . u-PAR molecule that is partly recovered in the medium and partly retained in the cells. In fact, cross-linking to iodinated **ATF** shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example. . .

DETD . . . expected to be unable to attach to the cell surface, to be secreted in the medium, and to bind pro-u-PA, **ATF**, DFP-u-PA and active u-PA, in general the same molecules bound by the normal u-PA receptor. It should therefore be useful. . .

DETD . . . in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the **amino terminal fragment** (**ATF**) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). **ATF**, u-PAR and DFP-inhibited u-PA were radio-labelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0.01% (v/v) Tween 80 in the case of **ATF** and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

DETD . . . M NaCl (pH 7.5) and the cells were washed twice with buffer A. In some experiments exogenously added ^{125} I-labelled DFP-**uPA** (1 nM) were allowed to rebind to the unoccupied u-PAR by incubation for 2 hours at 4° C. in buffer. . .

DETD . . . PI-PLC was essentially non-degraded and consisted primarily of intact two-chain u-PA (Mr 50,000) along with a smaller amount of its **amino terminal fragment** (**ATF**, Mr 17,000). The receptor-binding domain of u-PA resides in both of these components (Appella et al., 1987). Accordingly, these two. . . u-PA (Mr 33,000), devoid of the receptor-binding domain, was eliminated by the washing procedures. These data indicate that u-PA and **ATF** were released from the cell surface by PI-PLC, while they were specifically associated to u-PAR.

DETD . . . soluble protein (Mr 60,000) that still expressed high affinity towards ^{125} I-labelled DFP-u-PA (FIG. 14C) as well as ^{125} I-labelled **ATF** (data not shown). Furthermore, by SDS-PAGE and immunoblotting, a protein with similar Mr was detected in the serum-free medium after. . .

DETD . . . detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to, ^{125} I-labelled **ATF** (FIG. 15A), thus demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the **ATF**-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this. . .

DETD . . . detergents in the polyacrylamide gel (data not shown). This experiment shows that the PI-PLC induced change in phase-partitioning of the **ATF** binding activity is totally accounted for by an identical change in the hydrophobicity of the u-PAR protein itself.

DETD The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. ^{125} I-labelled aminoterminal fragment (**ATF**) of the **urokinase** were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells.

After increasing time of PMA treatment both an increase in the strength. . .

DETD . . . of u-PAR protein was studied by the cross linking assay as described. FIG. 20 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of dibuturyl cAMP treatment both an increase in the. . .

DETD . . . et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the proenzyme of **urokinase**-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum,. . . human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates **urokinase**-type plasminogen activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung,. . . IgG antibody to human u-PA (clone 2 (10 μg/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human **urokinase**-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic. . .

DETD . . . against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble **urokinase**.

DETD . . . Peeters, Ed.), 33, 623-626, 1985, and was a kind gift from E. Sarubbi and A. Soffientini. Two-chain u-PA and u-PA **amino-terminal fragment** (**ATF**) purification (Stoppelli et al., 1985) and DFP-treated u-PA preparation (Andreasen et al., 1986) have previously been described. Human plasmin (4. . .

DETD Iodinations. 1 μg portions of protein (**ATF**, u-PA or pro-u-PA) in 30 mM sodium phosphate buffer (pH 7.4) were iodinated with 1 mCi of Na^{125} I (Amersham. . .

DETD . . . (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for **ATF** and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4° C. After binding, the cells. . .

DETD Effect of u-PA and u-PA/PAI-1 Complex on Binding of ^{125} I-**ATF** to the u-PA Receptor

DETD In order to study the interaction between PAI-1 and receptor-bound u-PA, it was first tested whether purified PAI-1 competes with **ATF** for binding to the receptor on U937 cells, and it was found that it does not, event at a 1000:1 excess (data not shown). Then, the ability of unlabelled u-PA and preformed u-PA/PAI-1 complex to compete with ^{125} I-**ATF** for receptor binding was compared. FIGS. 29A-B show the dependence of the inhibition of ^{125} I-**ATF** binding to U937 cells on the concentration of unlabelled u-PA or u-PA/PAI-1 complex. Since PAI-1 forms stoichiometric covalent complexes with. . . presented in FIGS. 29A-B indicate that complexing of u-PA by PAI-1 does not dramatically alter its ability to compete with **ATF** for receptor binding. The slight difference in the shape of the competition curves, suggesting that u-PA is a 2-3 fold. . .

DETD . . . migration of the u-PA/PAI-1 complex. This band represents receptor-bound u-PA/PAI-1 complex as it is competed for by unlabelled 85 nM **ATF** or u-PA.

DETD . . . receptor was further investigated. u-PA binds the receptor through its amino-terminal extremity, and the binding is competed equally well by **ATF** or u-PA (Stoppelli et al., 1985). Accordingly, it was found that the binding of the u-PA/PAI-1 complex can be competed to the same extent by **ATF** and u-PA, with 50% competition reached around 1-2 nM (data not shown). Thus, even when complexed to its inhibitor, u-PA. . .

DETD . . . (step 1). In all cases, more than 90% of the binding occurring during step 1 was inhibited by u-PA or **ATF** while no inhibition was

obtained with low molecular weight u-PA, demonstrating the specificity of the interaction (data not shown). In. . .

DETD Four different iodinated ligands were tested: two-chain u-PA (u-PA), DFP-inactivated u-PA (DFP-u-PA), the **amino-terminal fragment** of u-PA (**ATF**) and the preformed u-PA:PAI-1 complex. The amount of receptor-bound ligand (cell-associated, acid-extracted radioactivity), of cell-trapped ligand (cell-associated, acid-resistant radioactivity) and. . . below). FIG. 33 shows the fate of the ligand during step 2 incubation at 37° C. In the case of **ATF** and DFP-u-PA, the receptor-bound fraction decreases slowly in agreement with previous data (Stoppelli et al., 1985); for u-PA, the decrease. . . little complex is found still to be surface-bound. The non-degraded, internalized ligand constitutes a small fraction in the case of **ATF**, but is clearly higher in all other cases. In particular in the case of the u-PA:PAI-1 complex, it increases rapidly. . . of the total radioactivity around 30 minutes, and decreasing thereafter. While very little ligand is degraded in the case of **ATF** and DFP-u-PA, a larger fraction is degraded in the case of u-PA (20% after 3 hours) and much more in. . . time course suggests a precursor-product relationship between the cell-trapped and the degraded ligand. Possibly, therefore, the u-PA:PAI-1 complex, but not **ATF** and DFP-u-PA, is internalized and then degraded. In the experiment shown in FIGS. 33A-D, u-PA might represent an intermediate case. . .

DETD The results unequivocally show that while **ATF**, DFP-treated u-PA and free active u-PA (in particular when excess low molecular weight u-PA is present to titrate endogenous inhibitors). . .

DETD Previous data have shown the absence of internalization of receptor-bound **ATF**, u-PA and pro-u-PA (Vassali et al., 1985; Stoppelli et al., 1985; Bajpai and Baker, 1985a; Stoppelli et al., 1986). These. . .

DETD . . . Plasminogen isoform 2 was used in all experiments described here. u-PA (M_r 55,000) was obtained either by plasmin activation of pro-**uPA** (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide. . . al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-**urokinase** (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The. . .

DETD . . . were then removed at various time points. The presence of u-PAR in these supernatants was demonstrated by cross-linking to ^{125} I-**ATF** using DSS as described in Example 1. The effect of this soluble form of u-PAR on u-PA enzymatic activity was. . .

DETD Supernatants from PI-PLC-treated PMA-stimulated U937 cells contain a soluble form of u-PAR, as determined by DSS crosslinking to ^{125} I-**ATF**. When these supernatants were incubated with u-PA, there was a concentration-dependent decrease in u-PA activity (FIG. 41) which was much. . . observed with the control supernatants, which is due to the small amounts of u-PAR observed in the sample by ^{125} I-**ATF** cross-linking.

DETD Mice of the BALB/c strain were immunized with u-PAR purified on a diisopropylfluoride **urokinase**-type plasminogen activator (DFP-u-PA) ligand affinity column. The mice were given three intraperitoneal injections with 5 μg of u-PAR with 3. . .

DETD 6) Blocking buffer: 25% fetal calf serum in PBS (25% FCS/PBS) or 1% skimmed milk **powder** (SMP) in PBS.

DETD . . . piece was lyophilized and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide **powder** was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit.. . .

DETD Assay for Inhibition of Cellular **ATF** Binding--U937 cells were washed and acid-treated, as described (Nielsen et al., 1988). The cells were

resuspended in 100 µl of. . . The samples were incubated for 1 hour at 4° C. with gentle stirring. After the incubation, 100 µl of ^{125} I-**ATF** was added and incubation was continued for another hour. In the 300-µl reaction volume, the final concentration of ^{125} I-**ATF** was 2.2 nM, and the final dilutions of anti-u-PAR serum/control serum ranged from 1:300 to 1:153,600. The cells were then. . .

DETD . . . was used in a competition experiment in which U937 cells were preincubated with the antiserum followed by addition of ^{125} I-**ATF**. As shown in FIGS. 45A-B, the anti-u-PAR serum was able to completely inhibit the specific binding of ^{125} I-**ATF** to the cells. 50% inhibition was obtained at a 1:2400 dilution. Under the same conditions, a control serum showed only. . .

DETD . . . serum (final IgG concentration 90 µg/ml during preincubation). This treatment completely hindered the subsequent formation of cross-linked conjugates with ^{125} I-**ATF**. The IgG from the pre-immune serum had no effect on the cross-linking assay at the same concentration.

DETD . . . at 37° C. This treatment led to an approx. 50% delipidation of u-PAR as judged by the shift of the **ATF** cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see Example 1).

DETD . . . S, Kristensen P, Gr.o slashed.ndahl-Hansen J, Skriver L, Dan.o slashed. K (1986) Plasminogen activator inhibitor from human fibrosarcoma cells binds **urokinase**-type plasminogen activator, but not its proenzyme. J Biol Chem 261: 7644-7651

DETD . . . Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani G, Blasi F (1987) The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

DETD Bajpai A, Baker J B (1985) Cryptic **urokinase** binding sites on human foreskin fibroblasts. Biochem Biophys Res Commun 133: 475-482

DETD Blasi F (1988) Surface receptors for **urokinase plasminogen activator**. Fibrinolysis 2: 73-84

DETD Blasi F, Stoppelli M P, Cubellis M V (1986) The receptor for **urokinase-plasminogen activator**. J Cell Biochem 32: 179-186

DETD Blasi F, Vassalli J-D, Dan.o slashed. K (1987) **Urokinase**-type plasminogen activator: proenzyme, receptor, and inhibitors. J Cell Biol 104: 801-804

DETD Boyd D, Florent G, Kim P, Brattain M (1988a) Determination of the levels of **urokinase** and its receptor in human colon carcinoma cell lines. Cancer Res 48: 3112-3116

DETD Boyd D, Florent G, Murano G, Brattain M (1988b) Modulation of the **urokinase** receptor in human colon cell lines by N,N-dimethylformamide. Biochim Biophys Acta 947: 96-100

DETD Collen D, Zamarron C, Lijnen H R, Hoylaerts M (1986) Activation of plasminogen by pro-**urokinase**. II. Kinetics. J Biol Chem 261: 1259-1266

DETD Cubellis M V, Nolli M L, Cassani G, Blasi F (1986) Binding of single-chain pro-**urokinase** to the **urokinase** receptor of human U937 cells. J Biol Chem 261: 15819-15822

DETD Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast **urokinase** proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

DETD Ellis V, Scully M F, Kakkar V V (1987) Plasminogen activation by single-chain **urokinase** in functional isolation. J Biol Chem 262: 14998-15003

DETD Ellis V, Scully M F, Kakkar V V (1988) Role of human U937 monocytes in controlling single-chain **urokinase**-initiated plasminogen activation. Fibrinolysis 2: supp. 1, 112

DETD Ellis V, Scully M F, Kakkar V V (1989) Plasminogen aktivation initiated by single-chain **urokinase**-type plasminogen activator. J Biol Chem 264, 2185-88

DETD Estreicher A, Wohlwend A, Belin D, Schleuning W-D, Vassalli J-D (1989) Characterization of the cellular binding site for the **urokinase**-type plasminogen activator. J Biol Chem 264: 1180-1189

DETD . . . Munkholm-Larsen P, Bach F, Nielsen L S, Dombernowsky P, Dan.o slashed. K (1988) Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. J Lab Clin Med 111: 42-51

DETD Gr.o slashed.ndahl-Hansen J, Lund L R, Ralfki.ae butted.r E, Ottevanger V, Dan.o slashed. K (1988) **Urokinase**- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. The Journal of Investigative Dermatology 90: 790-795

DETD . . . S, Kelley P, Suddith R L, Greenlee R (1984) Effective and fibrin-specific clot lysis by a zymogen precursor form of **urokinase** (pro-**urokinase**). A study In Vitro and in two animal species. J Clin Invest 73: 1731-1739

DETD Hearing V J, Law L W, Corti A, Appella E, Blasi F (1988) Modulation of metastatic potential by cell surface **urokinase** of murine melanoma cells. Cancer Res 48: 1270-1278

DETD Hebert C A, Baker J B (1988) Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: Colocalization of cell surface **urokinase** with vinculin. J Cell Biol 106: 1241-1247

DETD Janicke F, Schmitt M, Hafter A, Hollrieder A, Babic R, Ulm K, Gossner W, Graeff H (1990) **Urokinase**-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1-10

DETD Janicke F, Schmitt M, Ulm K, Gossner W, Graeff H (1989) **Urokinase**-type plasminogen activator antigen and early relapse in breast cancer. The Lancet, 1049

DETD Kasai S, Arimura H. Nishida M, Suyama T (1985) Proteolytic cleavage of single-chain pro-**urokinase** induces conformational change which follows activation of the zymogen and reduction of its high affinity for fibrin. J Biol Chem. . .

DETD Kielberg V, Andreasen P A, Gr.o slashed.ndahl-Hansen J, Nielsen L S, Skriver L, Dan.o slashed. K (1985) Proenzyme to **urokinase**-type plasminogen activator in the mouse in vivo. FEBS Lett 182: 441-445

DETD Lijnen H R, Zamarron C, Blaber M, Winkler M E, Collen D (1986) Activation of plasminogen by pro-**urokinase**. I. Mechanism. J Biol Chem 261: 1253-1258

DETD Miles L A, Plow E F (1987) Receptor mediated binding of the fibrinolytic components, plasminogen and **urokinase**, to peripheral blood cells. Thromb Haemostas 58: 936-942

DETD Needham G K, Sherbet G V, Farndon J R, Harris A L (1987) Binding of **urokinase** to specific receptor sites on human breast cancer membranes. Eur J Cancer 55: 13-16

DETD Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain **urokinase**-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689

DETD . . . S, Kellerman G M, Behrendt N, Picone R, Dan.o slashed. K, Blasi F (1988) A 55,000-60,000 M_r receptor protein for **urokinase**-type plasminogen activator, J Biol Chem 263: 2358-2363

DETD . . . A, Robbiati F, Soffientini A, Blasi F, Parenti F, Cassani G (1989) Production and characterization of human recombinant single chain **urokinase**-type plasminogen activator from mouse cells. Fibrinolysis 3: 101-106

DETD Pannell R, Gurewich V (1987) Activation of plasminogen by single-chain **urokinase** or by two-chain **urokinase**--a demonstration that single-chain **urokinase** has a low catalytic activity (pro-**urokinase**). Blood 69: 22-26

DETD Petersen L C, Lund L R, Nielsen L S, Dan.o slashed. K, Skriver L (1988) One-chain **urokinase**-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J Biol Chem 263: 11189-11195

DETD . . . N, Mastronicola M R, Cubellis M V, Stoppelli M P, Pedersen S,

Dan.o slashed. K, Blasi F (1989) Regulation of **urokinase** receptors in monocyte-like U937 cells by phorbol ester PMA. J Cell Biol 108: 693-702

DETD . . . F, Freaney D E, Plescia J, Miles L A (1986) The plasminogen system and cell surfaces: evidence for plasminogen and **urokinase** receptors on the same cell type. J Cell Biol 103: 2411-2420

DETD Pollanen J, Hedman K, Nielsen L S, Dan.o slashed. K, Vaheri A (1988) Ultra-structural localization of plasma membrane-associated **urokinase**-type plasminogen activator at focal contacts. J Cell Biol 106: 87-95

DETD Pollanen J, Saksela O, Salonen E-M, Andreasen P, Nielsen L S, Dan.o slashed. K, Vaheri A (1987) Distinct localizations of **urokinase**-type plasminogen activator and its type-1 inhibitor under cultured human fibroblasts and sarcoma cells. J Cell Biol 104: 1085-1096

DETD . . . L, Larsson L-I, Kielberg V, Nielsen L S, Andresen P B, Kristensen P, Dan.o slashed. K (1984) Immunocytochemical localization of **urokinase**-type plasminogen activator in Lewis lung carcinoma. J Cell Biol 99: 753-758

DETD Stephens R W, Alitalo R, Tapiovaara H, Vaheri A (1988) Production of an active **urokinase** by leukemia cell lines: a novel distinction from cell lines of solid tumors. Leukemia Res 12: 419-422

DETD Stephens R W, Fordham C J, Doe W F (1987) Proenzyme to **urokinase**-type plasminogen activator in human colon cancer: in vitro inhibition by monocyte minactivin after proteolytic activation. Eur J Cancer Clin Oncol. . .

DETD Stoppelli M P, Corti A, Soffientini A, Cassani G, Blasi F, Assoian R K (1985) Differentiation-enhanced binding of the **amino-terminal fragment** of human **urokinase plasminogen activator** to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA 82: 4939-4943

DETD . . . Tacchetti C, Cubellis M V, Corti A, Hearing V J, Cassani G, Appella E, Blasi F (1986) Autocrine saturation of pro-**urokinase** receptors on human A431 cells. Cell 45: 675-684

DETD Stump D C, Lijnen H R, Collen D (1986a) Purification and characterization of single-chain **urokinase**-type plasminogen activator from human cell cultures. J Biol Chem 261: 1274-1278

DETD Stump D C, Thienpont M, Collen D (1986b) **Urokinase**-related proteins in human urine. J Biol Chem 261: 1267-1273

DETD Thorsen S, Glas-Greenwalt P, Astrup T (1972) Difference in the binding to fibrin of **urokinase** and tissue plasminogen activator. Thrombos Diathes Haemorrh 28: 65-74

DETD Urano T, de Serrano VS, Gaffney PJ, Castellino FJ (1988) The activation of human (Glu^1) plasminogen by human single-chain **urokinase**. Arch Biochem Biophys 264: 222-230

DETD . . . J-D, Baccino D, Belin D (1985) A cellular binding site for the M_r 55,000 form of the human plasminogen activator, **urokinase**. J Cell Biol 100: 86-92

DETD Wun T-C. Ossowski L, Reich E (1982) A proenzyme form of human **urokinase**. J Biol Chem 157: 7262-7268

CLM What is claimed is:

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PAR) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting. . .

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .

5. A method according to claim 2 wherein the modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA).

8. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .
24. A method of inhibiting the binding of **urokinase** type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase plasminogen-activator** receptor (u-PAR) which comprises contacting a plasminogen-converting, receptor-binding form of u-PA or pro-u-PA, or a receptor (u-PAR) for said form. . .
26. A method of inhibiting the conversion of plasminogen to plasmin which comprises inhibiting the binding of a plasminogen-converting, **urokinase-plasminogen-activator** receptor by the method of claim 24, and hence inhibiting the consequent conversion of plasminogen to plasmin.

28. The method of claim 27 in which the substance is capable of inhibiting binding of u-PA or pro-**UPA** to u-PAR in a supernatant of HT-1080 cells.

```
=> s l9 and pharmaceutical
        195216 PHARMACEUTICAL
L10          1 L9 AND PHARMACEUTICAL

=> s l10 and (transnasal or intranasal or nasal)
           364 TRANSNASAL
         21681 INTRANASAL
         39685 NASAL
L11          1 L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)

=> d l11,kwic

L11  ANSWER 1 OF 1  USPATFULL on STN
TI     Urokinase-type plasminogen activator receptor
AI     US 1995-442108          19950516 (8)                          <--
AB     Activation of plasminogen to plasmin is inhibited by preventing the
       binding of a receptor binding form of urokinase-type plasminogen
       activator to a urokinase-type plasminogen activator receptor in a
       mammal, thereby preventing the urokinase-type plasminogen activator
       from converting plasminogen into plasmin. DNA fragments which encode for
       soluble, active fragments of the urokinase-type plasminogen activator
       receptor are provided.
SUMM   . . . the method comprising inhibiting the activation of plasminogen
       to plasmin by preventing the binding of a receptor binding form of
       urokinase-type plasminogen activator (in the following termed u-PA) to
       a u-PA receptor in the mammal and thereby preventing the u-PA from. .
       .
SUMM   According to the literature, urokinase-type plasminogen activator
       (u-PA) has been found in all mammalian species so far investigated.
       Several findings relate u-PA to tissue degradation. . .
SUMM   . . . in the primary structure is remote from the catalytic site. The
       receptor binding domain is located in the 15 kD amino-terminal
       fragment (ATF, residues 1-135) of the u-PA molecule, more precisely
       within the cysteine-rich region termed the growth factor region as this
       region. . .
SUMM   . . . al., 1985, Vassalli et al., 1985, Nielsen et al., 1988).
       Fragments of u-PA containing only the receptor binding domain, e.g.
       ATF, ensure specificity of the binding to the receptor, since other
       molecules that might bind u-PA (protease nexin and the specific. . .
```

SUMM . . . al., 1989, binding of the added single-chain u-PA to the receptor was prevented by preincubation of the cells with the **amino-terminal fragment** of u-PA. These experiments do not, therefore, as do the following examples, demonstrate displacement of endogenously produced u-PA, a prerequisite. . .

SUMM Human tumor cells are very commonly found to secrete plasminogen activator of the **urokinase** type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen. . .

SUMM . . . streptavidin-fluorescin isothiocyanate. The method is very sensitive, and its specificity can readily be tested by competition experiments (e.g. with the **amino-terminal fragment** of u-PA (**ATF**), t-PA, EGF, etc.).

SUMM While the present specification and claims relate predominantly to the **urokinase** type plasminogen activator (u-PA), it is obvious that the same approach can and should be used for tissue-type plasminogen activator. . .

SUMM . . . that the u-PA contains the u-PAR binding site. The receptor binding form of u-PA can thus be pro-u-PA, u-PA, an **amino-terminal fragment** of u-PA, a u-PA that is irreversibly inhibited by e.g. diisopropyl fluorophosphate (DFP), p-nitrophenyl-p'-guanidinobenzoate (NPGB), or any other inhibitor or. . .

SUMM The enzyme **urokinase**-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg^{560}, plasminogen is activated to the. . .

SUMM Another useful modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA) (cf. Stoppelli et al., 1985).

SUMM . . . by any administration method which is suitable for administering proteins or peptides or antibodies. Typical administration routes are parenteral, oral, **nasal**, topical or rectal administration. In each case, the active ingredient to be administered should be formulated in a manner which. . .

SUMM . . . by packing the active ingredient in such a way that it will not be released from the formulation (i.e. the **pharmaceutical** composition) until it has reached the site where either the active ingredient is to exert its activity locally (i.e. in. . .

SUMM **Nasal** administration is an administration form which is presently intensively investigated in order to provide absorption of substances of the peptide type from the **nasal** cavity. In principle, this may take place in two ways, firstly by using enhancers, and secondly by using the bioadhesion. . .

SUMM The **pharmaceutical** compositions of the invention may for example include pharmaceutically acceptable excipients adapted to the character of the active ingredients in accordance with the above discussion. Suitable excipients may include liposomes and/or microspheres. The preparation of the **pharmaceutical** compositions may be performed in accordance with methods described in the literature for compositions of the types described herein.

SUMM . . . mg such as about 150 mg for an average adult person. The same considerations apply with respect to NPGB-u-PA, the **amino-terminal fragment** of u-PA, and pro-u-PA that is modified so that it cannot be cleaved by plasmin. Evidently, the higher the affinity. . .

SUMM . . . of substrates which are useful in the present method as substrates for the enzymes mentioned above are H_2 O_2, p-nitrophenylphosphate, **lactose**, urea, β-D-glucose, CO_2, RNA, starch, or malate. The substrate may be combined with, e.g. a chromophore which is either a. . .

DRWD . . . of 100 nM: bovine serum albumin (lane 3), t-PA (lane 4), plasminogen (lane 5), murine epidermal growth factor (lane 6), **ATF** (lane 7), active 54 kD u-PA (lane 8), DFP-inactivated 54 kD u-PA (lane 9). After preincubation for 15 min at room temperature, ^{125}I-labelled **ATF** (approximately 1 nM) was added, followed by incubation

for 1 hour at 4° C. After incubation, chemical cross-linking was performed. . . by SDS-PAGE on a 6-16% gradient gel under non-reducing conditions and autoradiography. Lane 1 shows the cross-linked control with ^{125} I-**ATF** and no addition of u-PAR or competitors. Electrophoretic mobilities of molecular weight standard proteins are inducated (kD).

DRWD FIG. 3. Deglycosylation of cross-linked ^{125} I-**ATF**: u-PAR complexes from PMA-treated and nontreated U937a cells. PMA-treated (lanes 1 and 3) and nontreated (lanes 2 and 4) cells were acid-treated and lysed with 0.5% CHAPS. The lysates were incubated with ^{125} I-**ATF**, cross-linked with disuccinimidyl suberate, denatured under mildly reducing conditions, and then further incubated in the presence (lanes 3 and 4). . .

DRWD FIG. 5 shows chymotryptic fragments of u-PAR, analyzed by chemical cross-linking to ^{125} I-**ATF**. Preparation of samples and numbering of lanes are the same as in FIG. 4. The samples were 50-fold diluted and analyzed by chemical cross-linking to ^{125} I-**ATF**, followed by SDS-PAGE on a 6-16% gradient gel under reducing conditions, and autoradiography. The electrophoretic mobilities of molecular weight marker. . .

DRWD FIG. 6 shows deglycosylation of chymotryptic fragments, cross-linked to **ATF**. Samples of purified u-PAR were subjected to degradation with 8 ng/ml chymotrypsin (lanes 1 and 4) or 40 ng/ml chymotrypsin. . . incubated but received the same amount of phenylmethylsulfonylfluoride. The samples were 50-fold diluted, and subjected to chemical cross-linking to ^{125} I-**ATF**. The cross-linked samples were subjected to enzymatic deglycosylation with N-Glycanase (lanes 4-6) or treated in parallel without the addition of. . .

DRWD FIGS. 9A-9F. Caseinolytic plaque assay of **uPA** binding to LB6 cells transfected with p-uPAR-1 DNA. FIGS. 9A and C-F refer to clone LB6/p-uPAR-1 while plate B refers to clone LB6/RSVCAT. In plate A no **uPA** was added. Otherwise (FIGS. 9B-F) cells were subjected to a binding step with 0.2 nM human **uPA** for 1 hour at 37° C. The following competitors, present during the binding step, were used: none (FIGS. 9B, 9C); 100 nM **ATF** (FIG. 9D); 200 μM synthetic peptide human **uPA**[12-32(ala19)] (FIG. 9E); 100 μM synthetic peptide mouse **uPA**[13-33(ala20)] (FIG. 9F).

DRWD FIG. 10A. Binding of human ^{125} I-**ATF** to mouse LB6 cells transfected with RSVCAT (closed circles) and p-uPAR-1 DNA (closed circles). Specific binding was calculated by subtracting the counts not competed by 100 nM unlabelled **ATF** (about 1000 cpm in this experiment).

DRWD FIG. 10B. Reducing SDS-polyacrylamide (12.5%) gel electrophoretic analysis of the ^{125} I-**ATF** cross-linked to LB6/p-uPAR-1 cells. Lane 1 has the molecular weight markers (see Methods); lane 2 represents the migration of the labelled **ATF** (3,000 cpm). Lanes 3 and 4 show the migration of duplicate LB6/p-uPAR-1 extracts cross-linked with ligand. Lanes 5 and 6 show the competition of the cross-linking of LB6/p-uPAR-1 cells to the ligand by unlabelled **ATF** (100 nM final concentration). The last lane to the right shows the cross-linking obtained (in a separate experiment) with the. . .

DRWD FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated **ATF**, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as described before. . . Similarly, conditioned medium was centrifuged at 100,000×g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated **ATF**, cross-linked with DSS, and analyzed by SDS-PAGE (part B of the Figure). Lanes a and b are duplicates from cells. . .

DRWD . . . addition of extra Triton X-114 and 0.1 M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM ^{125} I-labelled **ATF** was performed on parallel aliquots of aqueous (A) and

detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to ^{125} I-**ATF**/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to ^{125} I-**ATF**. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to ^{125} I-**ATF**. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active **urokinase**. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal antibody to u-PA (anti-u-PA-ab). The bound. . .

DRWD FIGS. 29A-29B. Competition by unlabelled u-PA (.circle-solid.--.circle-solid.) or u-PA/PAI-1 complex (.smallcircle.--.smallcircle.) of the binding of ^{125} I-**ATF** to human U937 cells. [Competitor] is the concentration of free or PAI-1 complexed u-PA; for PAI-1/u-PA complex formation, a 50. . .

DRWD FIGS. 45A-45B. Inhibition of cellular **ATF** binding by antibodies raised against purified u-PAR. 5×10^5 U937a cells were preincubated with mouse antiserum raised against purified u-PAR (.circle-solid.--.circle-solid.). . . mouse antiserum raised against porcine mucins (.smallcircle.--.smallcircle.) for 1 hour at 4° C., followed by addition of 2.2 nM ^{125} I-**ATF** and incubation for another hour at the same temperature. The cells were then washed 3 times after which the cell-bound. . .

DETD . . . phosphate, 1.0 M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the ^{125} I-labelled amino terminal (**ATF**) fragment of **urokinase**, followed by SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH_2 -terminal amino acid sequencing were dialyzed. .

.

DETD Protein labelling with ^{125} I. ^{125} I-labelling of **ATF** was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80.. . .

DETD Chemical cross-linking assay. Cross-linking of u-PAR in complex mixtures or purified fractions to ^{125} I-labelled **ATF** was performed as described for solubilized receptor (Nielsen et al., 1988), except that 2 mM disuccinimidylsuberate (DSS) was used for. . .

DETD . . . in cell lysates and detergent fractions, the receptor was selectively labelled before the degradation by chemical cross-linking to ^{125} I-labelled **ATF**.

DETD For desialylation, 70 µl lysate samples labelled by cross-linking to ^{125} I-**ATF**, were made up to 200 µl with 0.05 M sodium acetate, pH 5.0. 90 µl aliquots of the mixture received. . .

DETD Analysis for binding activity toward the **ATF** of **urokinase** was performed by chemical cross-linking to ^{125} I-labelled **ATF** followed by SDS-PAGE and autoradiography. **ATF**-binding activity co-eluted with silver-stainable protein. The conjugate formed between **ATF** and the purified protein migrated as a 70-75 kDa component during electrophoresis (FIG. 1B, lane 2). As demonstrated previously for

partially purified u-PAR (Nielsen et al., 1988), the formed conjugate was indistinguishable from the cross-linked product formed with **ATF** on intact, PMA-stimulated U937 cells (not shown), as well as in non-purified detergent extracts from the same cells. Binding and cross-linking to ^{125} I-labelled **ATF** was specific and saturable. Thus, it could be competed for by an excess of unlabelled **ATF**, active u-PA or DFP-treated u-PA, while no competition was obtained with unrelated proteins such as, for example, bovine serum albumin,. . .

DETD . . . was performed with non-labelled components (FIG. 1C). In this experiment, DFP-treated u-PA was chosen as the u-PAR-specific ligand instead of **ATF**, since, because of the higher molecular weight, this ligand would lead to a conjugate clearly separable from the purified protein. . .

DETD . . . In these experiments, a selective labelling of u-PAR was performed before the deglycosylation reaction by chemical cross-linking to ^{125} I-labelled **amino terminal fragment** (**ATF**) of **urokinase** (Nielsen et al., 1988).

DETD . . . is seen (FIG. 3) that the cell lysates from which the receptor was purified gave rise to a 70-75 kDa u-PAR-**ATF** conjugate (lane 1) that could be deglycosylated to yield an approximately 50 kDa product (lane 3). **ATF** is known not to contain N-bound carbohydrate. Thus, as the change in apparent molecular weight was the same as that. . . protein above, this experiment provided independent evidence that the heavy glycosylation found is indeed a property of the only significant **ATF** binding component in the detergent lysates of these cells.

DETD When detergent lysates obtained from other cell lines were analyzed by chemical cross-linking to **ATF**, variations in the electrophoretic migration of the radiolabelled product were observed in certain cases. In these analyses, for comparison, individual. . .

DETD However, when samples representing the 4 patterns above were subjected to enzymatic deglycosylation after the cross-linking to ^{125} I-**ATF**, the molecular weight variation was abolished. The resulting conjugate band was sharp, and migrated as a 50 kDa component, irrespective. . .

DETD . . . detergent fractions from PMA-stimulated U937a cells led to an approximately 5 kDa reduction in the apparent molecular weight of the **ATF**-u-PAR conjugate. Thus, the glycosylation includes several sialic acid residues. The change in molecular weight, though undoubtedly present, appeared somewhat smaller. . .

DETD Samples to be analyzed by chemical cross-linking to ^{125} I-**ATF** were 50-fold diluted in 0.1 M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition. . .

DETD Deglycosylation of Samples, Cross-Linked to ^{125} I-**ATF**

DETD . . . of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or **ATF** as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further. . .

DETD In parallel, the samples were analyzed in the chemical cross-linking assay, using ^{125} I-**ATF** as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which. . . to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD **ATF**. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare. . .

DETD . . . a 16 kD product, consistent with the expected size for the fragment with binding activity observed after cross-linking to ^{125} I-**ATF**. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system, suggesting that this fragment does. . .

DETD . . . essential medium (DMEM) supplemented with 10% foetal calf serum, 2 mM glutamine and 10 IU/ml of penicillin and streptomycin. Human **high molecular weight urokinase** and prourokinase were provided by Lepetit SpA (Nolli et al., 1989). The **amino terminal fragment**

of human u-PA, **ATF**, was a gift from Abbott Laboratories. The synthetic peptides human u-PA[12-32(ala19)] and mouse u-PA[13-33(ala20)] have been described before (Appella et. . .

DETD . . . 9B), whereas those transfected with pRSVCAT DNA did not (see FIG. 9B). Specificity is shown by the ability of the **amino-terminal fragment** of u-PA (**ATF**), i.e. a truncated u-PA molecule maintaining the binding capacity but deprived of the catalytic activity (Stoppelli et al., 1985) (FIG.. . .

DETD . . . human u-PAR by mouse LB6 cells transfected with p-u-PAR-1 was further analysed by binding competition experiments using unlabelled and iodinated **ATF**. The molecular properties of the u-PAR expressed by the transfected cells were analysed by SDS-PACE and radiography of material from these cells cross-linked to iodinated **ATF**.

DETD Mouse LB6 cells were grown in DMEM as described in this Example. Iodination of **ATF** has been described previously by Stoppelli et al. (1985). The cross-linking reagent disuccinimidyl suberate was from Pierce Chemical Co.

DETD Binding of ^{125} I-**ATF**

DETD . . . bovine serum albumin, incubated in serum-free medium for 1 hour at 37° C., and then incubated with 47,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) at 37° C. for 60 minutes in the presence of different concentrations of unlabelled **ATF**. The experiment was carried out in duplicate. At the end of the incubation, the cells were washed with PBS-bovine serum. . . collected and counted (Stoppelli et al., 1985). Specific binding was calculated by subtracting the radioactivity not competed by 100 nM **ATF**.

DETD Cross-linking of ^{125} I-**ATF** to the u-PAR

DETD Cross-linking of LB6/p-u-PAR-1 cells with ^{125} I-**ATF** was carried out using disuccinimidyl suberate (DSS) as previously described (Picone et al., 1989). Duplicate dishes of 2.6×10^5 cells were washed with PBS-bovine serum albumin (1 mg/ml), incubated with 60,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) in serum-free DMEM supplemented with 25 mM Hepes, pH 7.4 for 60 minutes at 37° C., washed four. . . The cells were then lysed directly in Laemmli buffer containing 5% β-mercaptoethanol (Laemmli, 1970). In control samples, 100 nM unlabelled **ATF** was present during the binding step. The cell extract was analysed by SDS-polyacrylamide (12.5%) gel electrophoresis under reducing conditions (Laemmli,. . .

DETD Expression of p-u-PAR-1 DNA in LB6 cells is supported by quantitative binding data with ^{125} I-**ATF**. FIG. 10A shows a binding-competition plot in which control LB6 cells (LBS/RSVCAT) do not bind ^{125} I-**ATF**, whereas LB6 cells transfected with p-u-PAR-1 DNA do. The binding is specifically competed by unlabelled **ATF**. Scatchard plot of the data gave a Ka of about 10^8 moles^{-1} and about 25,000 receptors/cell.

DETD . . . has the correct molecular properties, cross-linking studies were performed with the LB6/p-u-PAR-1 cells. Cells were incubated with human ^{125} I-labelled **ATF**, bound **ATF** cross-linked with disuccinimidyl suberate, the cells lysed and analysed by SDS-polyacrylamide gel electrophoresis. The results are shown in FIG. 10B.. . . with human GM637 cells (from which the cDNA clone is derived). This is the molecular weight expected for the intact **ATF**-u-PAR complex (Nielsen et al., 1988). Considering the possible cell-dependent difference in glycosylation, and the fact that PMA-treated cells possess a. . .

DETD . . . the human u-PAR gene in mouse LB6 cells by the following findings: p-u-PAR-1 DNA transfected LB6 cells bind labelled human **ATF** and unlabelled human u-PA as shown by direct binding assay (FIG. 10A) and the caseinolytic plaque assay (FIG. 9). The binding is specific as shown by the ability of human **ATF**, human synthetic peptide u-PA[12-32(ala19)], but not mouse synthetic peptide u-PA[13-33(ala20)] to compete for binding (FIGS. 9A-F and 10A). The **ATF**-u-PAR complex has

the correct molecular weight (FIG. 10B).

DETD Production of a Soluble Receptor Protein Containing the Binding Site for **Urokinase**

DETD . . . u-PAR molecule that is partly recovered in the medium and partly retained in the cells. In fact, cross-linking to iodinated **ATF** shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example. . .

DETD . . . expected to be unable to attach to the cell surface, to be secreted in the medium, and to bind pro-u-PA, **ATF**, DFP-u-PA and active u-PA, in general the same molecules bound by the normal u-PA receptor. It should therefore be useful. . .

DETD . . . in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the **amino terminal fragment** (**ATF**) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). **ATF**, u-PAR and DFP-inhibited u-PA were radio-labelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0.01% (v/v) Tween 80 in the case of **ATF** and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

DETD . . . M NaCl (pH 7.5) and the cells were washed twice with buffer A. In some experiments exogenously added ^{125} I-labelled DFP-**uPA** (1 nM) were allowed to rebind to the unoccupied u-PAR by incubation for 2 hours at 4° C. in buffer. . .

DETD . . . PI-PLC was essentially non-degraded and consisted primarily of intact two-chain u-PA (Mr 50,000) along with a smaller amount of its **amino terminal fragment** (**ATF**, Mr 17,000). The receptor-binding domain of u-PA resides in both of these components (Appella et al., 1987). Accordingly, these two. . . u-PA (Mr 33,000), devoid of the receptor-binding domain, was eliminated by the washing procedures. These data indicate that u-PA and **ATF** were released from the cell surface by PI-PLC, while they were specifically associated to u-PAR.

DETD . . . soluble protein (Mr 60,000) that still expressed high affinity towards ^{125} I-labelled DFP-u-PA (FIG. 14C) as well as ^{125} I-labelled **ATF** (data not shown). Furthermore, by SDS-PAGE and immunoblotting, a protein with similar Mr was detected in the serum-free medium after. . .

DETD . . . detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to, ^{125} I-labelled **ATF** (FIG. 15A), thus demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the **ATF**-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this. . .

DETD . . . detergents in the polyacrylamide gel (data not shown). This experiment shows that the PI-PLC induced change in phase-partitioning of the **ATF** binding activity is totally accounted for by an identical change in the hydrophobicity of the u-PAR protein itself.

DETD The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. ^{125} I-labelled aminoterminal fragment (**ATF**) of the **urokinase** were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of PMA treatment both an increase in the strength. . .

DETD . . . of u-PAR protein was studied by the cross linking assay as described. FIG. 20 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of dibuturyl cAMP treatment both an increase in the. . .

DETD . . . et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the

proenzyme of **urokinase**-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum,. . . human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates **urokinase**-type plasminogen activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung,. . . IgG antibody to human u-PA (clone 2 (10 μg/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human **urokinase**-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic. . .

DETD . . . against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble **urokinase**.

DETD . . . Peeters, Ed.), 33, 623-626, 1985, and was a kind gift from E. Sarubbi and A. Soffientini. Two-chain u-PA and u-PA **amino-terminal fragment** (**ATF**) purification (Stoppelli et al., 1985) and DFP-treated u-PA preparation (Andreasen et al., 1986) have previously been described. Human plasmin (4. . .

DETD Iodinations. 1 μg portions of protein (**ATF**, u-PA or pro-u-PA) in 30 mM sodium phosphate buffer (pH 7.4) were iodinated with 1 mCi of Na^{125} I (Amersham. . .

DETD . . . (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for **ATF** and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4° C. After binding, the cells. . .

DETD Effect of u-PA and u-PA/PAI-1 Complex on Binding of ^{125} I-**ATF** to the u-PA Receptor

DETD In order to study the interaction between PAI-1 and receptor-bound u-PA, it was first tested whether purified PAI-1 competes with **ATF** for binding to the receptor on U937 cells, and it was found that it does not, event at a 1000:1 excess (data not shown). Then, the ability of unlabelled u-PA and preformed u-PA/PAI-1 complex to compete with ^{125} I-**ATF** for receptor binding was compared. FIGS. 29A-B show the dependence of the inhibition of ^{125} I-**ATF** binding to U937 cells on the concentration of unlabelled u-PA or u-PA/PAI-1 complex. Since PAI-1 forms stoichiometric covalent complexes with. . . presented in FIGS. 29A-B indicate that complexing of u-PA by PAI-1 does not dramatically alter its ability to compete with **ATF** for receptor binding. The slight difference in the shape of the competition curves, suggesting that u-PA is a 2-3 fold. . .

DETD . . . migration of the u-PA/PAI-1 complex. This band represents receptor-bound u-PA/PAI-1 complex as it is competed for by unlabelled 85 nM **ATF** or u-PA.

DETD . . . receptor was further investigated. u-PA binds the receptor through its amino-terminal extremity, and the binding is competed equally well by **ATF** or u-PA (Stoppelli et al., 1985). Accordingly, it was found that the binding of the u-PA/PAI-1 complex can be competed to the same extent by **ATF** and u-PA, with 50% competition reached around 1-2 nM (data not shown). Thus, even when complexed to its inhibitor, u-PA. . .

DETD . . . (step 1). In all cases, more than 90% of the binding occurring during step 1 was inhibited by u-PA or **ATF** while no inhibition was obtained with low molecular weight u-PA, demonstrating the specificity of the interaction (data not shown). In. . .

DETD Four different iodinated ligands were tested: two-chain u-PA (u-PA), DFP-inactivated u-PA (DFP-u-PA), the **amino-terminal fragment** of u-PA (**ATF**) and the preformed u-PA:PAI-1 complex. The amount of receptor-bound ligand (cell-associated, acid-extracted radioactivity), of cell-trapped ligand (cell-associated, acid-resistant radioactivity) and. . . below). FIG. 33 shows the fate of the ligand during step 2

incubation at 37° C. In the case of **ATF** and DFP-u-PA, the receptor-bound fraction decreases slowly in agreement with previous data (Stoppelli et al., 1985); for u-PA, the decrease. . . little complex is found still to be surface-bound. The non-degraded, internalized ligand constitutes a small fraction in the case of **ATF**, but is clearly higher in all other cases. In particular in the case of the u-PA:PAI-1 complex, it increases rapidly. . . of the total radioactivity around 30 minutes, and decreasing thereafter. While very little ligand is degraded in the case of **ATF** and DFP-u-PA, a larger fraction is degraded in the case of u-PA (20% after 3 hours) and much more in. . . time course suggests a precursor-product relationship between the cell-trapped and the degraded ligand. Possibly, therefore, the u-PA:PAI-1 complex, but not **ATF** and DFP-u-PA, is internalized and then degraded. In the experiment shown in FIGS. 33A-D, u-PA might represent an intermediate case. . .

DETD The results unequivocally show that while **ATF**, DFP-treated u-PA and free active u-PA (in particular when excess low molecular weight u-PA is present to titrate endogenous inhibitors). . .

DETD Previous data have shown the absence of internalization of receptor-bound **ATF**, u-PA and pro-u-PA (Vassali et al., 1985; Stoppelli et al., 1985; Bajpai and Baker, 1985a; Stoppelli et al., 1986). These. . .

DETD . . . Plasminogen isoform 2 was used in all experiments described here. u-PA (M_r 55,000) was obtained either by plasmin activation of pro-**uPA** (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide. . . al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-**urokinase** (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The. . .

DETD . . . were then removed at various time points. The presence of u-PAR in these supernatants was demonstrated by cross-linking to ^{125} I-**ATF** using DSS as described in Example 1. The effect of this soluble form of u-PAR on u-PA enzymatic activity was. . .

DETD Supernatants from PI-PLC-treated PMA-stimulated U937 cells contain a soluble form of u-PAR, as determined by DSS crosslinking to ^{125} I-**ATF**. When these supernatants were incubated with u-PA, there was a concentration-dependent decrease in u-PA activity (FIG. 41) which was much. . . observed with the control supernatants, which is due to the small amounts of u-PAR observed in the sample by ^{125} I-**ATF** cross-linking.

DETD Mice of the BALB/c strain were immunized with u-PAR purified on a diisopropylfluoride **urokinase**-type plasminogen activator (DFP-u-PA) ligand affinity column. The mice were given three intraperitoneal injections with 5 μg of u-PAR with 3. . .

DETD 6) Blocking buffer: 25% fetal calf serum in PBS (25% FCS/PBS) or 1% skimmed milk **powder** (SMP) in PBS.

DETD . . . piece was lyophilized and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide **powder** was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit.. . .

DETD Assay for Inhibition of Cellular **ATF** Binding--U937 cells were washed and acid-treated, as described (Nielsen et al., 1988). The cells were resuspended in 100 μl of. . . The samples were incubated for 1 hour at 4° C. with gentle stirring. After the incubation, 100 μl of ^{125} I-**ATF** was added and incubation was continued for another hour. In the 300-μl reaction volume, the final concentration of ^{125} I-**ATF** was 2.2 nM, and the final dilutions of anti-u-PAR serum/control serum ranged from 1:300 to 1:153,600. The cells were then. . .

DETD . . . was used in a competition experiment in which U937 cells were preincubated with the antiserum followed by addition of ^{125} I-**ATF**. As shown in FIGS. 45A-B, the anti-u-PAR serum was able to completely inhibit the specific binding of ^{125} I-**ATF** to the cells. 50% inhibition was obtained at a 1:2400 dilution. Under the same conditions, a control serum showed only. . .

DETD . . . serum (final IgG concentration 90 μg/ml during preincubation). This treatment completely hindered the subsequent formation of cross-linked conjugates with ^{125} I-**ATF**. The IgG from the pre-immune serum had no effect on the cross-linking assay at the same concentration.

DETD . . . at 37° C. This treatment led to an approx. 50% delipidation of u-PAR as judged by the shift of the **ATF** cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see Example 1).

DETD . . . S, Kristensen P, Gr.o slashed.ndahl-Hansen J, Skriver L, Dan.o slashed. K (1986) Plasminogen activator inhibitor from human fibrosarcoma cells binds **urokinase**-type plasminogen activator, but not its proenzyme. J Biol Chem 261: 7644-7651

DETD . . . Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani G, Blasi F (1987) The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

DETD Bajpai A, Baker J B (1985) Cryptic **urokinase** binding sites on human foreskin fibroblasts. Biochem Biophys Res Commun 133: 475-482

DETD Blasi F (1988) Surface receptors for **urokinase plasminogen activator**. Fibrinolysis 2: 73-84

DETD Blasi F, Stoppelli M P, Cubellis M V (1986) The receptor for **urokinase-plasminogen activator**. J Cell Biochem 32: 179-186

DETD Blasi F, Vassalli J-D, Dan.o slashed. K (1987) **Urokinase**-type plasminogen activator: proenzyme, receptor, and inhibitors. J Cell Biol 104: 801-804

DETD Boyd D, Florent G, Kim P, Brattain M (1988a) Determination of the levels of **urokinase** and its receptor in human colon carcinoma cell lines. Cancer Res 48: 3112-3116

DETD Boyd D, Florent G, Murano G, Brattain M (1988b) Modulation of the **urokinase** receptor in human colon cell lines by N,N-dimethylformamide. Biochim Biophys Acta 947: 96-100

DETD Collen D, Zamarron C, Lijnen H R, Hoylaerts M (1986) Activation of plasminogen by pro-**urokinase**. II. Kinetics. J Biol Chem 261: 1259-1266

DETD Cubellis M V, Nolli M L, Cassani G, Blasi F (1986) Binding of single-chain pro-**urokinase** to the **urokinase** receptor of human U937 cells. J Biol Chem 261: 15819-15822

DETD Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast **urokinase** proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

DETD Ellis V, Scully M F, Kakkar V V (1987) Plasminogen activation by single-chain **urokinase** in functional isolation. J Biol Chem 262: 14998-15003

DETD Ellis V, Scully M F, Kakkar V V (1988) Role of human U937 monocytes in controlling single-chain **urokinase**-initiated plasminogen activation. Fibrinolysis 2: supp. 1, 112

DETD Ellis V, Scully M F, Kakkar V V (1989) Plasminogen aktivation initiated by single-chain **urokinase**-type plasminogen activator. J Biol Chem 264, 2185-88

DETD Estreicher A, Wohlwend A, Belin D, Schleuning W-D, Vassalli J-D (1989) Characterization of the cellular binding site for the **urokinase**-type plasminogen activator. J Biol Chem 264: 1180-1189

DETD . . . Munkholm-Larsen P, Bach F, Nielsen L S, Dombernowsky P, Dan.o slashed. K (1988) Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. J Lab Clin Med 111: 42-51

DETD Gr.o slashed.ndahl-Hansen J, Lund L R, Ralfki.ae butted.r E, Ottevanger

V, Dan.o slashed. K (1988) **Urokinase**- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. The Journal of Investigative Dermatology 90: 790-795

DETD . . . S, Kelley P, Suddith R L, Greenlee R (1984) Effective and fibrin-specific clot lysis by a zymogen precursor form of **urokinase** (pro-**urokinase**). A study In Vitro and in two animal species. J Clin Invest 73: 1731-1739

DETD Hearing V J, Law L W, Corti A, Appella E, Blasi F (1988) Modulation of metastatic potential by cell surface **urokinase** of murine melanoma cells. Cancer Res 48: 1270-1278

DETD Hebert C A, Baker J B (1988) Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: Colocalization of cell surface **urokinase** with vinculin. J Cell Biol 106: 1241-1247

DETD Janicke F, Schmitt M, Hafter A, Hollrieder A, Babic R, Ulm K, Gossner W, Graeff H (1990) **Urokinase**-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1-10

DETD Janicke F, Schmitt M, Ulm K, Gossner W, Graeff H (1989) **Urokinase**-type plasminogen activator antigen and early relapse in breast cancer. The Lancet, 1049

DETD Kasai S, Arimura H. Nishida M, Suyama T (1985) Proteolytic cleavage of single-chain pro-**urokinase** induces conformational change which follows activation of the zymogen and reduction of its high affinity for fibrin. J Biol Chem. . .

DETD Kielberg V, Andreasen P A, Gr.o slashed.ndahl-Hansen J, Nielsen L S, Skriver L, Dan.o slashed. K (1985) Proenzyme to **urokinase**-type plasminogen activator in the mouse in vivo. FEBS Lett 182: 441-445

DETD Lijnen H R, Zamarron C, Blaber M, Winkler M E, Collen D (1986) Activation of plasminogen by pro-**urokinase**. I. Mechanism. J Biol Chem 261: 1253-1258

DETD Miles L A, Plow E F (1987) Receptor mediated binding of the fibrinolytic components, plasminogen and **urokinase**, to peripheral blood cells. Thromb Haemostas 58: 936-942

DETD Needham G K, Sherbet G V, Farndon J R, Harris A L (1987) Binding of **urokinase** to specific receptor sites on human breast cancer membranes. Eur J Cancer 55: 13-16

DETD Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain **urokinase**-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689

DETD . . . S, Kellerman G M, Behrendt N, Picone R, Dan.o slashed. K, Blasi F (1988) A 55,000-60,000 M_r receptor protein for **urokinase**-type plasminogen activator, J Biol Chem 263: 2358-2363

DETD . . . A, Robbiati F, Soffientini A, Blasi F, Parenti F, Cassani G (1989) Production and characterization of human recombinant single chain **urokinase**-type plasminogen activator from mouse cells. Fibrinolysis 3: 101-106

DETD Pannell R, Gurewich V (1987) Activation of plasminogen by single-chain **urokinase** or by two-chain **urokinase**--a demonstration that single-chain **urokinase** has a low catalytic activity (pro-**urokinase**). Blood 69: 22-26

DETD Petersen L C, Lund L R, Nielsen L S, Dan.o slashed. K, Skriver L (1988) One-chain **urokinase**-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J Biol Chem 263: 11189-11195

DETD . . . N, Mastronicola M R, Cubellis M V, Stoppelli M P, Pedersen S, Dan.o slashed. K, Blasi F (1989) Regulation of **urokinase** receptors in monocyte-like U937 cells by phorbol ester PMA. J Cell Biol 108: 693-702

DETD . . . F, Freaney D E, Plescia J, Miles L A (1986) The plasminogen system and cell surfaces: evidence for plasminogen and **urokinase** receptors on the same cell type. J Cell Biol 103: 2411-2420

DETD Pollanen J, Hedman K, Nielsen L S, Dan.o slashed. K, Vaheri A (1988) Ultra-structural localization of plasma membrane-associated **urokinase**-type plasminogen activator at focal contacts. J Cell Biol

106: 87-95

DETD Pollanen J, Saksela O, Salonen E-M, Andreasen P, Nielsen L S, Dan.o slashed. K, Vaheri A (1987) Distinct localizations of **urokinase**-type plasminogen activator and its type-1 inhibitor under cultured human fibroblasts and sarcoma cells. J Cell Biol 104: 1085-1096

DETD . . . L, Larsson L-I, Kielberg V, Nielsen L S, Andresen P B, Kristensen P, Dan.o slashed. K (1984) Immunocytochemical localization of **urokinase**-type plasminogen activator in Lewis lung carcinoma. J Cell Biol 99: 753-758

DETD Stephens R W, Alitalo R, Tapiovaara H, Vaheri A (1988) Production of an active **urokinase** by leukemia cell lines: a novel distinction from cell lines of solid tumors. Leukemia Res 12: 419-422

DETD Stephens R W, Fordham C J, Doe W F (1987) Proenzyme to **urokinase**-type plasminogen activator in human colon cancer: in vitro inhibition by monocyte minactivin after proteolytic activation. Eur J Cancer Clin Oncol. . .

DETD Stoppelli M P, Corti A, Soffientini A, Cassani G, Blasi F, Assoian R K (1985) Differentiation-enhanced binding of the **amino-terminal fragment** of human **urokinase plasminogen activator** to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA 82: 4939-4943

DETD . . . Tacchetti C, Cubellis M V, Corti A, Hearing V J, Cassani G, Appella E, Blasi F (1986) Autocrine saturation of pro-**urokinase** receptors on human A431 cells. Cell 45: 675-684

DETD Stump D C, Lijnen H R, Collen D (1986a) Purification and characterization of single-chain **urokinase**-type plasminogen activator from human cell cultures. J Biol Chem 261: 1274-1278

DETD Stump D C, Thienpont M, Collen D (1986b) **Urokinase**-related proteins in human urine. J Biol Chem 261: 1267-1273

DETD Thorsen S, Glas-Greenwalt P, Astrup T (1972) Difference in the binding to fibrin of **urokinase** and tissue plasminogen activator. Thrombos Diathes Haemorrh 28: 65-74

DETD Urano T, de Serrano VS, Gaffney PJ, Castellino FJ (1988) The activation of human (Glu^1) plasminogen by human single-chain **urokinase**. Arch Biochem Biophys 264: 222-230

DETD . . . J-D, Baccino D, Belin D (1985) A cellular binding site for the M_r 55,000 form of the human plasminogen activator, **urokinase**. J Cell Biol 100: 86-92

DETD Wun T-C. Ossowski L, Reich E (1982) A proenzyme form of human **urokinase**. J Biol Chem 157: 7262-7268

CLM What is claimed is:

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PAR) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from converting. . .

. . . comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .

5. A method according to claim 2 wherein the modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA).

8. A method for preventing or counteracting localized extracellular proteolytic activity in a mammal, comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a plasminogen converting receptor binding form of **urokinase**-type plasminogen activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase**-type plasminogen activator receptor (u-PA receptor) in the mammal and thereby preventing said form of u-PA or its proenzyme (pro-u-PA) from. . .

24. A method of inhibiting the binding of **urokinase** type plasminogen

activator (u-PA) or its proenzyme (pro-u-PA) to a **urokinase plasminogen-activator** receptor (u-PAR) which comprises contacting a plasminogen-converting, receptor-binding form of u-PA or pro-u-PA, or a receptor (u-PAR) for said form. . .
26. A method of inhibiting the conversion of plasminogen to plasmin which comprises inhibiting the binding of a plasminogen-converting, **urokinase-plasminogen-activator** receptor by the method of claim 24, and hence inhibiting the consequent conversion of plasminogen to plasmin.

28. The method of claim 27 in which the substance is capable of inhibiting binding of u-PA or pro-**UPA** to u-PAR in a supernatant of HT-1080 cells.

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     FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1          9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2           231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3           106 S L2 AND AD<MAR 01 2001
L4             8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)
L5            25 S L3 AND POWDER
L6            19 S L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L7             1 S L4 AND L6
L8             1 S L7 AND (POWDER)
L9             1 S L8 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L10            1 S L9 AND PHARMACEUTICAL
L11            1 S L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)

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         58622 LYOPHILIZ?
L12         18 L5 AND LYOPHILIZ?

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           364 TRANSNASAL
         21681 INTRANASAL
         39685 NASAL
L13         13 L12 AND (TRANSNASAL OR INTRANASAL OR NASAL)

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L14         13 L13 AND PHARMACEUTICAL?

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L14  ANSWER 1 OF 13  USPATFULL on STN
TI     Urokinase-type plasminogen activator receptor

L14  ANSWER 2 OF 13  USPATFULL on STN
TI     β-sheet mimetics and methods relating to the use thereof

L14  ANSWER 3 OF 13  USPATFULL on STN
TI     Expression of urokinase plasminogen activator inhibitors

L14  ANSWER 4 OF 13  USPATFULL on STN
TI     Method of treating a urokinase-type plasminogen activator-mediated
       disorder

L14  ANSWER 5 OF 13  USPATFULL on STN
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TI **Urokinase**-type plasminogen activator receptor

L14 ANSWER 6 OF 13 USPATFULL on STN
TI **Urokinase** receptor ligands

L14 ANSWER 7 OF 13 USPATFULL on STN
TI Methods for regulating transcription factors

L14 ANSWER 8 OF 13 USPATFULL on STN
TI Peptide analog inhibitors of **urokinase** receptor activity

L14 ANSWER 9 OF 13 USPATFULL on STN
TI Vectors and methods for recombinant production of **uPA**-binding fragments of the human **urokinase**-type plasminogen receptor (uPAR)

L14 ANSWER 10 OF 13 USPATFULL on STN
TI **Urokinase** receptor ligands

L14 ANSWER 11 OF 13 USPATFULL on STN
TI Oligonucleotides encloding peptide inhibitors of **urokinase** receptor activity

L14 ANSWER 12 OF 13 USPATFULL on STN
TI Peptide inhibitors of **urokinase** receptor activity

L14 ANSWER 13 OF 13 USPATFULL on STN
TI Branched combinatorial libraries

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L14 ANSWER 9 OF 13 USPATFULL on STN
1999:43412 Vectors and methods for recombinant production of **uPA**-binding fragments of the human **urokinase**-type plasminogen receptor (uPAR).
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Napoli, Italy
Masucci, Maria Teresa, Napoli, Italy
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Schleuning, Wolf-Dieter, Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
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Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Helsinki, Finland
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Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 5891664 19990406
APPLICATION: US 1994-319052 19941006 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Vectors and methods for recombinant production of **uPA**-binding fragments of the human **urokinase**-type plasminogen receptor (uPAR)

AI US 1994-319052 19941006 (8) <--

AB Activation of plasminogen to plasma is inhibited by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator to a **urokinase**-type plasminogen activator receptor in a mammal, thereby preventing the **urokinase**-type plasminogen activator from converting plasminogen into plasmin. DNA fragments which encode for soluble, active fragments of the **urokinase**-type plasminogen activator are provided.

SUMM . . . the method comprising inhibiting the activation of plasminogen to plasmin by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator (in the following termed u-PA) to a u-PA receptor in the mammal and thereby preventing the u-PA from. . .

SUMM According to the literature, **urokinase**-type plasminogen activator (u-PA) has been found in all mammalian species so far investigated. Several findings relate u-PA to tissue degradation. . .

SUMM . . . in the primary structure is remote from the catalytic site. The receptor binding domain is located in the 15 kD **amino-terminal fragment** (**ATF**, residues 1-135) of the u-PA molecule, more precisely within the cysteine-rich region termed the growth factor region as this region. . .

SUMM . . . al., 1985, Vassalli et al., 1985, Nielsen et al., 1988). Fragments of u-PA containing only the receptor binding domain, e.g. **ATF**, ensure specificity of the binding to the receptor, since other molecules that might bind u-PA (protease nexin and the specific. . .

SUMM . . . al., 1989, binding of the added single-chain u-PA to the receptor was prevented by preincubation of the cells with the **amino-terminal fragment** of u-PA. These experiments do not, therefore, as do the following examples, demonstrate displacement of endogenously produced u-PA, a prerequisite. . .

SUMM Human tumor cells are very commonly found to secrete plasminogen activator of the **urokinase** type (u-PA). By this means they are able to recruit the proteolytic potential available in the high concentration of plasminogen. . .

SUMM . . . streptavidin-fluorescin isothiocyanate. The method is very sensitive, and its specificity can readily be tested by competition experiments (e.g. with the **amino-terminal fragment** of u-PA (**ATF**), t-PA, EGF, etc.).

SUMM While the present specification and claims relate predominantly to the **urokinase** type plasminogen activator (u-PA), it is obvious that the same approach can and should be used for tissue-type plasminogen activator. . .

SUMM . . . that the u-PA contains the u-PAR binding site. The receptor binding form of u-PA can thus be pro-u-PA, u-PA, an **amino-terminal fragment** of u-PA, a u-PA that is irreversibly inhibited by e.g. diisopropyl fluorophosphate (DFP), p-nitrophenyl-p'-guanidinobenzoate (NPGB), or any other inhibitor or. . .

SUMM The enzyme **urokinase**-type plasminogen activator (u-PA) has only one well-defined macromolecular substrate, namely plasminogen. By cleavage at Arg^{560}, plasminogen is activated to the. . .

SUMM Another useful modification of u-PA is an **amino-terminal fragment** of u-PA (**ATF**-u-PA) (cf. Stoppelli et al., 1985).

SUMM . . . by any administration method which is suitable for administering proteins or peptides or antibodies. Typical administration routes are parenteral, oral, **nasal**, topical or rectal administration. In each case, the active ingredient to be administered should be formulated in a manner which. . .

SUMM . . . by packing the active ingredient in such a way that it will not be released from the formulation (i.e. the **pharmaceutical** composition) until it has reached the site where either the active ingredient is to exert its activity locally (i.e. in. . .

SUMM **Nasal** administration is an administration form which is presently intensively investigated in order to provide absorption of substances of

the peptide type from the **nasal** cavity. In principle, this may take place in two ways, firstly by using enhancers, and secondly by using the bioadhesion. . .

SUMM The **pharmaceutical** compositions of the invention may for example include **pharmaceutically** acceptable excipients adapted to the character of the active ingredients in accordance with the above discussion. Suitable excipients may include liposomes and/or microspheres. The preparation of the **pharmaceutical** compositions may be performed in accordance with methods described in the literature for compositions of the types described herein.

SUMM . . . mg such as about 150 mg for an average adult person. The same considerations apply with respect to NPGB-u-PA, the **amino-terminal fragment** of u-PA, and pro-u-PA that is modified so that it cannot be cleaved by plasmin. Evidently, the higher the affinity. . .

DRWD . . . to affinity chromatography using immobilized DFP-treated u-PA. The neutralized column eluate was dialyzed aginst 0.1% acetic acid and concentrated by **lyophilization**. A portion, representing 2×10^8 cells before purification, was run on 6-16% gradient SDS-PAGE under reducing conditions (lane 1). The gel. . .

DRWD . . . of 100 nM: bovine serum albumin (lane 3), t-PA (lane 4), plasminogen (lane 5), murine epidermal growth factor (lane 6), **ATF** (lane 7), active 54 kD u-PA (lane 8), DFP-inactivated 54 kD u-PA (lane 9). After preincubation for 15 min at room temperature, ^{125} I-labelled **ATF** (approximately 1 nM) was added, followed by incubation for 1 hour at 4° C. After incubation, chemical cross-linking was performed. . . by SDS-PAGE on a 6-16% gradient gel under non-reducing conditions and autoradiography. Lane 1 shows the cross-linked control with ^{125} I-**ATF** and no addition of u-PAR or competitors. Electrophoretic mobilities of molecular weight standard proteins are inducated (kD).

DRWD FIG. 3. Deglycosylation of cross-linked ^{125} I-**ATF**: u-PAR complexes from PMA-treated and nontreated U937a cells. PMA-treated (lanes 1 and 3) and nontreated (lanes 2 and 4) cells were acid-treated and lysed with 0.5% CHAPS. The lysates were incubated with ^{125} I-**ATF**, cross-linked with disuccinimidyl suberate, denatured under mildly reducing conditions, and then further incubated in the presence (lanes 3 and 4). . .

DRWD FIG. 5 shows chymotryptic fragments of u-PAR, analyzed by chemical cross-linking to ^{125} I-**ATF**. Preparation of samples and numbering of lanes are the same as in FIG. 4. The samples were 50-fold diluted and analyzed by chemical cross-linking to ^{125} I-**ATF**, followed by SDS-PAGE on a 6-16% gradient gel under reducing conditions, and autoradiography. The electrophoretic mobilities of molecular weight marker. . .

DRWD FIG. 6 shows deglycosylation of chymotryptic fragments, cross-linked to **ATF**. Samples of purified u-PAR were subjected to degradation with 8 ng/ml chymotrypsin (lanes 1 and 4) or 40 ng/ml chymotrypsin. . . incubated but received the same amount of phenylmethylsulfonylfluoride. The samples were 50-fold diluted, and subjected to chemical cross-linking to ^{125} I-**ATF**. The cross-linked samples were subjected to enzymatic deglycosylation with N-Glycanase (lanes 4-6) or treated in parallel without the addition of. . .

DRWD FIGS. 9A-9F. Caseinolytic plaque assay of **uPA** binding to LB6 cells transfected with p-uPAR-1 DNA. FIGS. 9A and C-F refer to clone LB6/p-uPAR-1 while plate B refers to clone LB6/RSVCAT. In plate A no **uPA** was added. Otherwise (FIGS. 9B-F) cells were subjected to a binding step with 0.2 nM human **uPA** for 1 hour at 37° C. The following competitors, present during the binding step, were used: none (FIGS. 9B, C); 100 nM **ATF** (FIG. 9D); 200 μM synthetic peptide human **uPA**[12-32(ala19)] (FIG. 9E); 100 μM synthetic peptide mouse **uPA**[13-33(ala20)] (FIG. 9F).

DRWD FIG. 10A. Binding of human ^{125} I-**ATF** to mouse LB6 cells

transfected with RSVCAT (closed circles) and p-uPAR-1 DNA (closed circles). Specific binding was calculated by subtracting the counts not competed by 100 nM unlabelled **ATF** (about 1000 cpm in this experiment).

DRWD FIG. 10B. Reducing SDS-polyacrylamide (12.5%) gel electrophoretic analysis of the ^{125} I-**ATF** cross-linked to LB6/p-uPAR-1 cells. Lane 1 has the molecular weight markers (see Methods); lane 2 represents the migration of the labelled **ATF** (3,000 cpm). Lanes 3 and 4 show the migration of duplicate LB6/p-uPAR-1 extracts cross-linked with ligand. Lanes 5 and 6 show the competition of the cross-linking of LB6/p-uPAR-1 cells to the ligand by unlabelled **ATF** (100 nM final concentration). The last lane to the right shows the cross-linking obtained (in a separate experiment) with the. . .

DRWD FIG. 11 shows SDS-PAGE (12.5%) electrophoretic analysis of the p-u-PAR-PFLM-1 mutant transfected into LB6 cells. Cells were incubated with iodinated **ATF**, washed, extracted with Triton X-114, and an amount of extract corresponding to 300,000 cells cross-linked with DSS as described before. . . Similarly, conditioned medium was centrifuged at 100,000×g, and the supernatant (a volume corresponding to 15,000 cells) was incubated with iodinated **ATF**, cross-linked with DSS, and analyzed by SDS-PAGE (left-hand panel). Lanes a and b are duplicates from cells grown at different. . .

DRWD . . . interference on amino acid analysis from low molecular weight compounds, this receptor preparation was dialysed thoroughly against 0.1% acetic acid, **lyophilized** and then subjected to Tricine-SDS-PAGE followed by electrotransfer onto a 0,45 μm PVDF-membrane (8 cm×8 cm). The insert shows the. . . mobility of u-PAR was observed in this experiment, due to a large excess of the zwitterionic detergent CHAPS in the **lyophilized** preparation. The stained area of the PVDF-membrane representing u-PAR was excised and hydrolysed in vacuo for 20 hours at 110°. . .

DRWD . . . by addition of extra Triton X-114 and 0.1M Tris (pH 8.1), respectively. Finally, cross-linking analysis with 1 nM ^{125} I-labelled **ATF** was performed on parallel aliquots of aqueous (A) and detergent (D) phases, followed by SDS-PAGE (10% T and 2.5% C) under non-reducing conditions. Areas corresponding to ^{125} I-**ATF**/u-PAR complexes (Mr 70,000) were excised from the polyacrylamide gel and the radioactivity was determined (shown as % of total radioactivity. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with PMA for different time periods, chemical cross linked to ^{125} I-**ATF**. Non-treated cells and PMA (150 nM) treated cells were acid treated and lysed. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . from Triton X-114 phase-separated extracts from U937 cells treated with Dibuturyl cAMP for different time periods, chemical cross-linked to ^{125} I-**ATF**. Non-treated cells and Dibuturyl cAMP (1 mM) treated cells were acid treated and lysed as described in Materials and Methods. The detergent phases were incubated with ^{125} I-**ATF**, cross linked with DSS and run in a 6-16% SDS-PAGE gradient gel followed by autoradiography. Electrophoretic mobility of molecular weight. . .

DRWD . . . presence of tranexamic acid), plasmin (pl, closed rectangles) is formed on the cell by the action of the bound active **urokinase**. This step may be inhibited by PAI-1 and PAI-2, and by an anti-catalytic monoclonal antibody to u-PA (anti-u-PA-ab). The bound. . .

DRWD FIG. 29. Competition by unlabelled u-PA (.circle-solid.--.circle-solid.) or u-PA/PAI-1 complex (.smallcircle.--.smallcircle.) of the binding of ^{125} I-**ATF** to human U937 cells. [Competitor] is the concentration of free or PAI-1 complexed u-PA; for PAI-1/u-PA complex formation, a 50. . .

DRWD FIG. 45. Inhibition of cellular **ATF** binding by antibodies raised

against purified u-PAR. 5×10^5 U937a cells were preincubated with mouse antiserum raised against purified u-PAR (.circle-solid.-.circle-solid.). . . mouse antiserum raised against porcine mucins (.smallcircle.-.smallcircle.) for 1 hour at 4° C., followed by addition of 2.2 nM ^{125} I-**ATF** and incubation for another hour at the same temperature. The cells were then washed 3 times after which the cell-bound. . .

DETD . . . sodium phosphate, 1.0M sodium carbonate, pH 9.0. u-PAR-containing fractions were identified by chemical cross-linking to the ^{125} I-labelled amino terminal (**ATF**) fragment of **urokinase**, followed by SDS-PAGE and autoradiography. Purified u-PAR samples for amino acid analysis or NH_2 -terminal amino acid sequencing were dialyzed against 0.1% acetic acid and **lyophilized**.

DETD Protein labelling with ^{125} I. ^{125} I-labelling of **ATF** was performed as described previously (Nielsen et al., 1988), except that 0.1% Triton X100 was replaced by 0.01% Tween 80.. . .

DETD Chemical cross-linking assay. Cross-linking of u-PAR in complex mixtures or purified fractions to ^{125} I-labelled **ATF** was performed as described for solubilized receptor (Nielsen et al., 1988), except that 2 mM disuccinimidylsuberate (DSS) was used for. . .

DETD . . . in cell lysates and detergent fractions, the receptor was selectively labelled before the degradation by chemical cross-linking to ^{125} I-labelled **ATF**.

DETD **Lyophilized**, purified u-PAR was radioiodinated directly.

DETD For desialylation, 70 μl lysate samples labelled by cross-linking to ^{125} I-**ATF**, were made up to 200 μl with 0.05M sodium acetate, pH 5.0. 90 μl aliquots of the mixture received either. . .

DETD . . . chromatography. The acid eluates were neutralized and analyzed, either directly or after concentration by dialysis against 0.1% acetic acid and **lyophilization**. The electrophoretic appearance of the purified material is shown in FIGS. 1A-C.

DETD Analysis for binding activity toward the **ATF** of **urokinase** was performed by chemical cross-linking to ^{125} I-labelled **ATF** followed by SDS-PAGE and autoradiography. **ATF**-binding activity co-eluted with silver-stainable protein. The conjugate formed between **ATF** and the purified protein migrated as a 70-75 kDa component during electrophoresis (FIG. 1B, lane 2). As demonstrated previously for partially purified u-PAR (Nielsen et al., 1988), the formed conjugate was indistinguishable from the cross-linked product formed with **ATF** on intact, PMA-stimulated U937 cells (not shown), as well as in non-purified detergent extracts from the same cells. Binding and cross-linking to ^{125} I-labelled **ATF** was specific and saturable. Thus, it could be competed for by an excess of unlabelled **ATF**, active u-PA or DFP-treated u-PA, while no competition was obtained with unrelated proteins such as, for example, bovine serum albumin,. . .

DETD . . . was performed with non-labelled components (FIG. 1C). In this experiment, DFP-treated u-PA was chosen as the u-PAR-specific ligand instead of **ATF**, since, because of the higher molecular weight, this ligand would lead to a conjugate clearly separable from the purified protein. . .

DETD . . . experiments were performed. In the first sequencing experiment, direct NH_2 -terminal sequencing of affinity-purified u-PAR was performed after dialysis and **lyophilization**. A partial sequence (Table 2A) was obtained, and it was demonstrated that only one sequence was present in the purified. . .

DETD In the second sequencing experiment, dialyzed and **lyophilized**, purified u-PAR was subjected to Tricine-SDS-PAGE, electroblotted onto a PVDF-membrane, Coomassie-stained, alkylated, and excised as described above, and then subjected. . .

DETD . . . In these experiments, a selective labelling of u-PAR was performed before the deglycosylation reaction by chemical cross-linking to ^{125} I-labelled **amino terminal fragment** (**ATF**) of

urokinase (Nielsen et al., 1988).

DETD . . . is seen (FIG. 3) that the cell lysates from which the receptor was purified gave rise to a 70-75 kDa u-PAR-**ATF** conjugate (lane 1) that could be deglycosylated to yield an approximately 50 kDa product (lane 3). **ATF** is known not to contain N-bound carbohydrate. Thus, as the change in apparent molecular weight was the same as that. . . protein above, this experiment provided independent evidence that the heavy glycosylation found is indeed a property of the only significant **ATF** binding component in the detergent lysates of these cells.

DETD When detergent lysates obtained from other cell lines were analyzed by chemical cross-linking to **ATF**, variations in the electrophoretic migration of the radiolabelled product were observed in certain cases. In these analyses, for comparison, individual. . .

DETD However, when samples representing the 4 patterns above were subjected to enzymatic deglycosylation after the cross-linking to ^{125} I-**ATF**, the molecular weight variation was abolished. The resulting conjugate band was sharp, and migrated as a 50 kDa component, irrespective. . .

DETD . . . detergent fractions from PMA-stimulated U937a cells led to an approximately 5 kDa reduction in the apparent molecular weight of the **ATF**-u-PAR conjugate. Thus, the glycosylation includes several sialic acid residues. The change in molecular weight, though undoubtedly present, appeared somewhat smaller. . .

DETD . . . Where footnotes are present, they indicate the best guess.

A. Direct sequencing of affinity purified u-PAR after dialysis against 0.1M acetic acid and **lyophilization**. The initial yield was 70 pmol PTH-Leu at step 1. Note that direct sequencing does not allow the identification of cysteine residues.

Res.. . .

DETD Enzymatic degradation: Affinity purified u-PAR was dialyzed against 0.1% acetic acid and **lyophilized** as described in Example 1. The freeze-dried material was redissolved in incubation buffer (0.05M Tris/HCl, 0.05% CHAPS, pH 8.1) to. . .

DETD Samples to be analyzed by chemical cross-linking to ^{125} I-**ATF** were 50-fold diluted in 0.1M Tris/HCl, 1% Triton X-114, pH 8.1. The diluted samples were either clarified by addition of. . .

DETD Deglycosylation of samples, cross-linked to ^{125} I-**ATF**

DETD . . . of u-PAR (see "Results" below) to the binding domain of the receptor requires a cross-linking experiment using non-labelled DFP-u-PA or **ATF** as the ligand and analysis by SDS-PAGE and silver staining, using the metods already adopted (see Example 1). For further. . .

DETD In parallel, the samples were analyzed in the chemical cross-linking assay, using ^{125} I-**ATF** as the ligand (FIG. 5). While the non-degraded samples (lanes 4 and 5) showed the 70-75 kD conjugate band which. . . to be expected for a conjugate formed between the above mentioned, 16 kD u-PAR degradation product and the 15 kD **ATF**. The presence of a minor binding activity corresponding to intact u-PAR was ascribed to the cleavage being slightly incomplete; compare. . .

DETD . . . a 16 kD product, consistent with the expected size for the fragment with binding activity observed after cross-linking to ^{125} I-**ATF**. Unlike the intact u-PAR, the ligand binding fragment proved hydrophilic in the Triton X-114 system, suggesting that this fragment does. . .

DETD . . . essential medium (DMEM) supplemented with 10% fetal calf serum, 2 mM glutamine and 10 IU/ml of penicillin and streptomycin. Human **high molecular weight urokinase** and prourokinase were provided by Lepetit SpA (Nolli et al., 1989). The **amino terminal fragment** of human u-PA, **ATF**, was a gift from Abbott Laboratories. The synthetic peptides human u-PA[12-32(ala19)] and mouse u-PA[13-33(ala20)] have been described before (Appella et. . .

DETD . . . 9B), whereas those transfected with pRSVCAT DNA did not (see

FIG. 9B). Specificity is shown by the ability of the **amino-terminal fragment** of u-PA (**ATF**), i.e. a truncated u-PA molecule maintaining the binding capacity but deprived of the catalytic activity (Stoppelli et al., 1985) (FIG.. . .

DETD . . . human u-PAR by mouse LB6 cells transfected with p-u-PAR-1 was further analysed by binding competition experiments using unlabelled and iodinated **ATF**. The molecular properties of the u-PAR expressed by the transfected cells were analysed by SDS-PAGE and radiography of material from these cells cross-linked to iodinated **ATF**.

DETD Mouse LB6 cells were grown in DMEM as described in this Example. Iodination of **ATF** has been described previously by Stoppelli et al. (1985). The cross-linking reagent disuccinimidyl suberate was from Pierce Chemical Co.

DETD Binding of ^{125} I-**ATF**

DETD . . . bovine serum albumin, incubated in serum-free medium for 1 hour at 37° C., and then incubated with 47,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) at 37° C. for 60 minutes in the presence of different concentrations of unlabelled **ATF**. The experiment was carried out in duplicate. At the end of the incubation, the cells were washed with PBS-bovine serum. . . collected and counted (Stoppelli et al., 1985). Specific binding was calculated by subtracting the radioactivity not competed by 100 nM **ATF**.

DETD Cross-linking of ^{125} I-**ATF** to the u-PAR

DETD Cross-linking of LB6/p-u-PAR-1 cells with ^{125} I-**ATF** was carried out using disuccinimidyl suberate (DSS) as previously described (Picone et al., 1989). Duplicate dishes of 2.6×10^5 cells were washed with PBS-bovine serum albumin (1 mg/ml), incubated with 60,000 cpm ^{125} I-**ATF** (1500 cpm/fmole) in serum-free DMEM supplemented with 25 mM Hepes, pH 7.4 for 60 minutes at 37° C., washed four. . . The cells were then lysed directly in Laemmli buffer containing 5% β-mercaptoethanol (Laemmli, 1970). In control samples, 100 nM unlabelled **ATF** was present during the binding step. The cell extract was analysed by SDS-polyacrylamide (12.5%) gel electrophoresis under reducing conditions (Laemmli,. . .

DETD Expression of p-u-PAR-1 DNA in LB6 cells is supported by quantitative binding data with ^{125} I-**ATF**. FIG. 10A shows a binding-competition plot in which control LB6 cells (LBS/RSVCAT) do not bind ^{125} I-**ATF**, whereas LB6 cells transfected with p-u-PAR-1 DNA do. The binding is specifically competed by unlabelled **ATF**. Scatchard plot of the data gave a Ka of about 10^8 moles^{-1} and about 25,000 receptors/cell.

DETD . . . has the correct molecular properties, cross-linking studies were performed with the LB6/p-u-PAR-1 cells. Cells were incubated with human ^{125} I-labelled **ATF**, bound **ATF** cross-linked with disuccinimidyl suberate, the cells lysed and analysed by SDS-polyacrylamide gel electrophoresis. The results are shown in FIG. 10B.. . . with human GM637 cells (from which the cDNA clone is derived). This is the molecular weight expected for the intact **ATF**-u-PAR complex (Nielsen et al., 1988). Considering the possible cell-dependent difference in glycosylation, and the fact that PMA-treated cells possess a. . .

DETD . . . the human u-PAR gene in mouse LB6 cells by the following findings: p-u-PAR-1 DNA transfected LB6 cells bind labelled human **ATF** and unlabelled human u-PA as shown by direct binding assay (FIG. 10A) and the caseinolytic plaque assay (FIG. 9). The binding is specific as shown by the ability of human **ATF**, human synthetic peptide u-PA[12-32(ala19)], but not mouse synthetic peptide u-PA[13-33(ala20)] to compete for binding (FIGS. 9A-F 10A). The **ATF**-u-PAR complex has the correct molecular weight (FIG. 10B).

DETD Production of a Soluble Receptor Protein Containing the Binding Site for **Urokinase**

DETD . . . u-PAR molecule that is partly recovered in the medium and

partly retained in the cells. In fact, cross-linking to iodinated **ATF** shows a single band in the medium and two bands in the Triton X-114 extract (prepared as described in Example. . .

DETD . . . expected to be unable to attach to the cell surface, to be secreted in the medium, and to bind pro-u-PA, **ATF**, DFP-u-PA and active u-PA, in general the same molecules bound by the normal u-PA receptor. It should therefore be useful. . .

DETD . . . in Example 1. Active human u-PA was purchaged from Serono and was DFP-inactivated as described (Nielsen et al., 1988); the **amino terminal fragment** (**ATF**) of u-PA was a kind gift from Dr. G. Cassani (LePetit, Italy). **ATF**, u-PAR and DFP-inhibited u-PA were radiolabelled as described (Nielsen et al., 1988) except that 0.1% (v/v) Triton X-100 was replaced by 0.1% (w/v) CHAPS in the case of u-PAR and by 0,01% (v/v) Tween 80 in the case of **ATF** and DFP-u-PA. Preparation of polyclonal rabbit antibodies against human u-PAR was carried out as described in Example 11.

DETD . . . 0.1M NaCl (pH 7.5) and the cells were washed twice with buffer A. In some experiments exogenously added ^{125} I-labelled DFP-**uPA** (1 nM) were allowed to rebind to the unoccupied u-PAR by incubation for 2 hours at 4° C. in buffer. . .

DETD . . . 0.5M Tris, 0.1% (w/v) SDS and 12 mM 3-mercaptopropionic acid (added as scavenger) for 4 hours at 15 mA/gel. Purified, **lyophilized** u-PAR was reduced by boiling for 2 minutes in 4% (w/v) SDS, 12% (w/v) glycerol, 50 mM Tris and 40. . .

DETD . . . PI-PLC was essentially non-degraded and consisted primarily of intact two-chain u-PA (Mr 50,000) along with a smaller amount of its **amino terminal fragment** (**ATF**, Mr 17,000). The receptor-binding domain of u-PA resides in both of these components (Appella et al., 1987). Accordingly, these two. . . u-PA (Mr 33,000), devoid of the receptor-binding domain, was eliminated by the washing procedures. These data indicate that u-PA and **ATF** were released from the cell surface by PI-PLC, while they were specifically associated to u-PAR.

DETD . . . soluble protein (Mr 60,000) that still expressed high affinity towards ^{125} I-labelled DFP-u-PA (FIG. 14C) as well as ^{125} I-labelled **ATF** (data not shown). Furthermore, by SDS-PAGE and immunoblotting, a protein with similar Mr was detected in the serum-free medium after. . .

DETD . . . detergent-phase separation by Triton X-114, it almost quantitatively partitioned into the detergent phase, as assessed by cross-linking to ^{125} I-labelled **ATF** (FIG. 15A), thus demonstrating the very hydrophobic properties of the receptor. Incubation with PI-PLC altered the hydrophobicity of the u-PA binding protein substantially, as more than 50% of the **ATF**-binding activity was now recovered in the aqueous phase (FIG. 15B). It proved impossible to achieve a higher level of this. . .

DETD . . . detergents in the polyacrylamide gel (data not shown). This experiment shows that the PI-PLC induced change in phase-partitioning of the **ATF** binding activity is totally accounted for by an identical change in the hydrophobicity of the u-PAR protein itself.

DETD The effect of PMA on production of u-PAR protein was studied by cross-linking experiment. ^{125} I-labelled aminoterminal fragment (**ATF**) of the **urokinase** were chemically cross linked to the detergent phase of phase-separated Triton X-114 extracts prepared from U937 cells treated with PMA for different time periods. FIG. 18 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of PMA treatment both an increase in the strength. . .

DETD . . . of u-PAR protein was studied by the cross linking assay as described. FIG. 20 shows a weak signal of ^{125} I-**ATF** cross-linked to the u-PAR in control U937 cells. After increasing time of dibuturyl cAMP treatment both an increase in the. . .

DETD . . . et al., "Monoclonal antibodies inhibitory to human plasmin: definitive demonstration of a role for plasmin in activating the

proenzyme of **urokinase**-type plasminogen activator", Eur. J. Biochem. 158: 537-542, 1986); aprotinin (Trasylol, Bayer, Leverkusen, FRG; 200 KIU/ml); tranexamic acid (Cyclokapron, Kabi Vitrum,. . . human type-2 plasminogen activator inhibitor minactivin (see Golder, J. P. et al., "Minactivin: A human monocyte product which specifically inactivates **urokinase**-type plasminogen activators", Eur. J. Biochem. 136: 517-522, 1983), PAI-2 purified from cultures of human U-937 histiocytic lymphoma cells (see Leung,. . . IgG antibody to human u-PA (clone 2 (10 µg/ml) in Nielsen, L. S. et al., "Enzyme-linked immunosorbent assay for human **urokinase**-type plasminogen activators and its proenzyme using a combination of monoclonal and polyclonal antibodies", J. Immunoassay 7: 209-228, 1986); the anti-catalytic. . .

DETD . . . against Tris/Tween. No residual DFP inhibitor could be detected when the preparation was tested in an activity assay of soluble **urokinase**.

DETD . . . Peeters, Ed.), 33, 623-626, 1985, and was a kind gift from E. Sarubbi and A. Soffientini. Two-chain u-PA and u-PA **amino-terminal fragment** (**ATF**) purification (Stoppelli et al., 1985) and DFP-treated u-PA preparation (Andreasen et al., 1986) have previously been described. Human plasmin (4. . .

DETD Iodinations. 1 µg portions of protein (**ATF**, u-PA or pro-u-PA) in 30 mM sodium phosphate buffer (pH 7.4) were iodinated with 1 mCi of Na^{125} I (Amersham. . .

DETD . . . (phosphate buffered saline supplemented with 0.1% bovine serum albumin) containing iodinated ligands (about 50,000 cpm corresponding to 0.1 nM for **ATF** and 0.05 nM for pro-u-PA and u-PA) and incubated for the indicated time at 4° C. After binding, the cells. . .

DETD . . . with 10 volumes of buffer and eluted with 1M NaCl in 0.1M acetic acid (pH 2.7), dialysed against 0.03% SDS, **lyophilized** and subjected to SDS-gel electrophoresis (see above).

DETD Effect of u-PA and u-PA/PAI-1 complex on binding of ^{125} I-**ATF** to the u-PA receptor

DETD In order to study the interaction between PAI-1 and receptor-bound u-PA, it was first tested whether purified PAI-1 competes with **ATF** for binding to the receptor on U937 cells, and it was found that it does not, event at a 1000:1 excess (data not shown). Then, the ability of unlabelled u-PA and preformed u-PA/PAI-1 complex to compete with ^{125} I-**ATF** for receptor binding was compared. FIGS. 29A-B shows the dependence of the inhibition of ^{125} I-**ATF** binding to U937 cells on the concentration of unlabelled u-PA or u-PA/PAI-1 complex. Since PAI-1 forms stoichiometric covalent complexes with. . . presented in FIG. 29 indicate that complexing of u-PA by PAI-1 does not dramatically alter its ability to compete with **ATF** for receptor binding. The slight difference in the shape of the competition curves, suggesting that u-PA is a 2-3 fold. . .

DETD . . . migration of the u-PA/PAI-1 complex. This band represents receptor-bound u-PA/PAI-1 complex as it is competed for by unlabelled 85 nM **ATF** or u-PA.

DETD . . . receptor was further investigated. u-PA binds the receptor through its amino-terminal extremity, and the binding is competed equally well by **ATF** or u-PA (Stoppelli et al., 1985). Accordingly, it was found that the binding of the u-PA/PAI-1 complex can be competed to the same extent by **ATF** and u-PA, with 50% competition reached around 1-2 nM (data not shown). Thus, even when complexed to its inhibitor, u-PA. . .

DETD . . . (step 1). In all cases, more than 90% of the binding occurring during step 1 was inhibited by u-PA or **ATF** while no inhibition was obtained with low molecular weight u-PA, demonstrating the specificity of the interaction (data not shown). In. . .

DETD Four different iodinated ligands were tested: two-chain u-PA (u-PA), DFP-inactivated u-PA (DFP-u-PA), the **amino-terminal fragment** of u-PA (**ATF**) and the preformed u-PA:PAI-1 complex. The amount of

receptor-bound ligand (cell-associated, acid-extracted radioactivity), of cell-trapped ligand (cell-associated, acid-resistant radioactivity) and. . . below). FIG. 33 shows the fate of the ligand during step 2 incubation at 37° C. In the case of **ATF** and DFP-u-PA, the receptor-bound fraction decreases slowly in agreement with previous data (Stoppelli et al., 1985); for u-PA, the decrease. . . little complex is found still to be surface-bound. The non-degraded, internalized ligand constitutes a small fraction in the case of **ATF**, but is clearly higher in all other cases. In particular in the case of the u-PA:PAI-1 complex, it increases rapidly. . . of the total radioactivity around 30 minutes, and decreasing thereafter. While very little ligand is degraded in the case of **ATF** and DFP-u-PA, a larger fraction is degraded in the case of u-PA (20% after 3 hours) and much more in. . . time course suggests a precursor-product relationship between the cell-trapped and the degraded ligand. Possibly, therefore, the u-PA:PAI-1 complex, but not **ATF** and DFP-u-PA, is internalized and then degraded. In the experiment shown in FIGS. 33A-D, u-PA might represent an intermediate case. . .

DETD The results unequivocally show that while **ATF**, DFP-treated u-PA and free active u-PA (in particular when excess low molecular weight u-PA is present to titrate endogenous inhibitors). . .

DETD Previous data have shown the absence of internalization of receptor-bound **ATF**, u-PA and pro-u-PA (Vassali et al., 1985; Stoppelli et al., 1985; Bajpai and Baker, 1985a; Stoppelli et al., 1986). These. . .

DETD . . . Plasminogen isoform 2 was used in all experiments described here. u-PA (M_r 55,000) was obtained either by plasmin activation of pro-**uPA** (Ellis et al., 1987) or as Ukidan (Serono). Both preparations were greater than 95% high molecular weight u-PA by SDS-polyacrylamide. . . al., 1986. Active PAI-1 was purified from the serum-free conditioned medium of Hep G2 cells by affinity chromatography on immobilized anhydro-**urokinase** (Wun et al., 1989). PAI-2 was purified from U937 cell lysates by chromatofocusing as described (Kruithof et al., 1986). The. . .

DETD . . . were then removed at various time points. The presence of u-PAR in these supernatants was demonstrated by cross-linking to ^{125} I-**ATF** using DSS as described in Example 1. The effect of this soluble form of u-PAR on u-PA enzymatic activity was. . .

DETD Supernatants from PI-PLC-treated PMA-stimulated U937 cells contain a soluble form of u-PAR, as determined by DSS crosslinking to ^{125} 1-**ATF**. When these supernatants were incubated with u-PA, there was a concentration-dependent decrease in u-PA activity (FIG. 41) which was much. . . observed with the control supernatants, which is due to the small amounts of u-PAR observed in the sample by ^{125} I-**ATF** cross-linking.

DETD Mice of the BALB/c strain were immunized with u-PAR purified on a diisopropylfluoride **urokinase**-type plasminogen activator (DFP-u-PA) ligand affinity column. The mice were given three intraperitoneal injections with 5 μg of u-PAR with 3. . .

DETD 6) Blocking buffer: 25% fetal calf serum in PBS (25% FCS/PBS) or 1% skimmed milk **powder** (SMP) in PBS.

DETD . . . molecular weight markers run in neighbouring lanes, the electrophoretic region corresponding to the antigen was excised. The gel piece was **lyophilized** and subsequently macerated in a Mikro-Dismembrator II apparatus (B. Braun AG, Federal Republic of Germany). The polyacrylamide **powder** was reconstituted in Tris-buffered saline, mixed with Freund's incomplete adjuvant and used for injection of a New Zealand white rabbit.. . .

DETD Assay for Inhibition of Cellular **ATF** Binding--U937 cells were washed and acid-treated, as described (Nielsen et al., 1988). The cells were resuspended in 100 μl of. . . The samples were incubated for 1 hour at 4° C. with gentle stirring. After the incubation, 100

µl of ^{125} I-**ATF** was added and incubation was continued for another hour. In the 300-µl reaction volume, the final concentration of ^{125} I-**ATF** was 2.2 nM, and the final dilutions of anti-u-PAR serum/control serum ranged from 1:300 to 1:153,600. The cells were then. . .

DETD . . . was used in a competition experiment in which U937 cells were preincubated with the antiserum followed by addition of ^{125} I-**ATF**. As shown in FIG. 45, the anti-u-PAR serum was able to completely inhibit the specific binding of ^{125} I-**ATF** to the cells. 50% inhibition was obtained at a 1:2400 dilution. Under the same conditions, a control serum showed only. . .

DETD . . . serum (final IgG concentration 90 µg/ml during preincubation). This treatment completely hindered the subsequent formation of crosslinked conjugates with ^{125} I-**ATF**. The IgG from the pre-immune serum had no effect on the cross-linking assay at the same concentration.

DETD . . . at 37° C. This treatment led to an approx. 50% delipidation of u-PAR as judged by the shift of the **ATF** cross-linking activity towards the buffer phase in the Triton X114 phase separation system (see Example 1).

DETD . . . S, Kristensen P, Gr.o slashed.ndahl-Hansen J, Skriver L, Dan.o slashed. K (1986) Plasminogen activator inhibitor from human fibrosarcoma cells binds **urokinase**-type plasminogen activator, but not its proenzyme. J Biol Chem 261: 7644-7651

DETD . . . Robinson E A, Ullrich S J, Stoppelli M P, Corti A, Cassani C, Blasi F (1987) The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. J Biol Chem 262: 4437-4440

DETD Bajpai A, Baker J B (1985) Cryptic **urokinase** binding sites on human foreskin fibroblasts. Biochem Biophys Res Commun 133: 475-482

DETD Blasi F (1988) Surface receptors for **urokinase plasminogen activator**. Fibrinolysis 2: 73-84

DETD Blasi F, Stoppelli M P, Cubellis M V (1986) The receptor for **urokinase-plasminogen activator**. J Cell Biochem 32: 179-186

DETD Blasi F, Vassalli J-D, Dan.o slashed. K (1987) **Urokinase**-type plasminogen activator: proenzyme, receptor, and inhibitors. J Cell Biol 104: 801-804

DETD Boyd D, Florent G, Kim P, Brattain M (1988a) Determination of the levels of **urokinase** and its receptor in human colon carcinoma cell lines. Cancer Res 48: 3112-3116

DETD Boyd D, Florent G, Murano G, Brattain M (1988b) Modulation of the **urokinase** receptor in human colon cell lines by N,N-dimethylformamide. Biochim Biophys Acta 947: 96-100

DETD Collen D, Zamarron C, Lijnen H R, Hoylaerts M (1986) Activation of plasminogen by pro-**urokinase**. II. Kinetics. J Biol Chem 261: 1259-1266

DETD Cubellis M V, Nolli M L, Cassani G, Blasi F (1986) Binding of single-chain pro-**urokinase** to the **urokinase** receptor of human U937 cells. J Biol Chem 261: 15819-15822

DETD Eaton D L, Scott R W, Baker J B (1984) Purification of human fibroblast **urokinase** proenzyme and analysis of its regulation by proteases and protease nexin. J Biol Chem 259: 6241-6247

DETD Ellis V, Scully M F, Kakkar V V (1987) Plasminogen activation by single-chain **urokinase** in functional isolation. J Biol Chem 262: 14998-15003

DETD Ellis V, Scully M F, Kakkar V V (1988) Role of human U937 monocytes in controlling single-chain **urokinase**-initiated plasminogen activation. Fibrinolysis 2: supp. 1, 112

DETD Ellis V, Scully M F, Kakkar V V (1989) Plasminogen aktivation initiated by single-chain **urokinase**-type plasminogen activator. J Biol Chem 264, 2185-88

DETD Estreicher A, Wohlwend A, Belin D, Schleuning W-D, Vassalli J-D (1989) Characterization of the cellular binding site for the **urokinase**-type

plasminogen activator. J Biol Chem 264: 1180-1189
DETD . . . Munkholm-Larsen P, Bach F, Nielsen L S, Dombernowsky P, Dan.o slashed. K (1988) Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. J Lab Clin Med 111: 42-51
DETD Gr.o slashed.ndahl-Hansen J, Lund L R, Ralfk.ae butted.r E, Ottevanger V, Dan.o slashed. K (1988) **Urokinase**- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. The Journal of Investigative Dermatology 90: 790-795
DETD . . . S, Kelley P, Suddith R L, Greenlee R (1984) Effective and fibrin-specific clot lysis by a zymogen precursor form of **urokinase** (pro-**urokinase**). A study In Vitro and in two animal species. J Clin Invest 73: 1731-1739
DETD Hearing V J, Law L W, Corti A, Appella E, Blasi F (1988) Modulation of metastatic potential by cell surface **urokinase** of murine melanoma cells. Cancer Res 48: 1270-1278
DETD Hebert C A, Baker J B (1988) Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: Colocalization of cell surface **urokinase** with vinculin. J Cell Biol 106: 1241-1247
DETD Janicke F, Schmitt M, Hafter A, Hollrieder A, Babic R, Ulm K, Gossner W, Graeff H (1990) **Urokinase**-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1-10
DETD Janicke F, Schmitt M, Ulm K, Gossner W, Graeff H (1989) **Urokinase**-type plasminogen activator antigen and early relapse in breast cancer. The Lancet, 1049
DETD Kasai S, Arimura H, Nishida M, Suyama T (1985) Proteolytic cleavage of single-chain pro-**urokinase** induces conformational change which follows activation of the zymogen and reduction of its high affinity for fibrin. J Biol Chem. . .
DETD Kielberg V, Andreasen P A, Gr.o slashed.ndahl-Hansen J, Nielsen L S, Skriver L, Dan.o slashed. K (1985) Proenzyme to **urokinase**-type plasminogen activator in the mouse In vivo. FEBS Lett 182: 441-445
DETD Lijnen H R, Zamarron C, Blaber M, Winkler H E, Collen D (1986) Activation of plasminogen by pro-**urokinase**. I. Mechanism. J Biol Chem 261: 1253-1258
DETD Miles L A, Plow E F (1987) Receptor mediated binding of the fibrinolytic components, plasminogen and **urokinase**, to peripheral blood cells. Thromb Haemostas 58: 936-942
DETD Needham G K, Sherbet G V, Farndon J R, Harris A L (1987) Binding of **urokinase** to specific receptor sites on human breast cancer membranes. Eur J Cancer 55: 13-16
DETD Nelles L, Lijnen H R, Collen D, Holmes W E (1987) Characterization of recombinant human single chain **urokinase**-type plasminogen activator mutants produced by site-specific mutagenesis of lysine 158. J Biol Chem 262: 5682-5689
DETD . . . S, Kellerman G M, Behrendt N, Picone R, Dan.o slashed. K, Blasi F (1988) A 55,000-60,000 M_r receptor protein for **urokinase**-type plasminogen activator. J Biol Chem 263: 2358-2363
DETD . . . A, Robbiati F, Soffientini A, Blasi F, Parenti F, Cassani C (1989) Production and characterization of human recombinant single chain **urokinase**-type plasminogen activator from mouse cells. Fibrinolysis 3: 101-106
DETD Pannell R, Gurewich V (1987) Activation of plasminogen by single-chain **urokinase** or by two-chain **urokinase**--a demonstration that single-chain **urokinase** has a low catalytic activity (pro-**urokinase**). Blood 69: 22-26
DETD Petersen L C, Lund L R, Nielsen L S, Dan.o slashed. K, Skriver L (1988) One-chain **urokinase**-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. J Biol Chem 263: 11189-11195
DETD . . . N, Mastronicola M R, Cubellis M V, Stoppelli M P, Pedersen S, Dan.o slashed. K, Blasi F (1989) Regulation of **urokinase** receptors in monocyte-like U937 cells by phorbol ester PMA. J Cell Biol 108: 693-702

DETD . . . F, Freaney D E, Plescia J, Miles L A (1986) The plasminogen system and cell surfaces: evidence for plasminogen and **urokinase** receptors on the same cell type. J Cell Biol 103: 2411-2420

DETD Pollanen J, Hedman K, Nielsen L S, Dan.o slashed. K, Vaheri A (1988) Ultrastructural localization of plasma membrane-associated **urokinase**-type plasminogen activator at focal contacts. J Cell Biol 106: 87-95

DETD Pollanen J, Saksela O, Salonen E-M, Andreasen P, Nielsen L S, Dan.o slashed. K, Vaheri A (1987) Distinct localizations of **urokinase**-type plasminogen activator and its type-1 inhibitor under cultured human fibroblasts and sarcoma cells. J Cell Biol 104: 1085-1096

DETD . . . L, Larsson L-I, Kielberg V, Nielsen L S, Andresen P B, Kristensen P, Dan.o slashed. K (1984) Immunocytochemical localization of **urokinase**-type plasminogen activator in Lewis lung carcinoma. J Cell Biol 99: 753-758

DETD Stephens R W, Alitalo R, Tapiovaara H, Vaheri A (1988) Production of an active **urokinase** by leukemia cell lines: a novel distinction from cell lines of solid tumors. Leukemia Res 12: 419-422

DETD Stephens R W, Fordham C J, Doe W F (1987) Proenzyme to **urokinase**-type plasminogen activator in human colon cancer: in vitro inhibition by monocyte minactivin after proteolytic activation. Eur J Cancer Clin Oncol. . .

DETD Stoppelli M P, Corti A, Soffientini A, Cassani G, Blasi F, Assoian R K (1985) Differentiation-enhanced binding of the **amino-terminal fragment** of human **urokinase plasminogen activator** to a specific receptor on U937 monocytes. Proc Natl Acad Sci USA 82: 4939-4943

DETD . . . Tacchetti C, Cubellis M V, Corti A, Hearing V J, Cassani G, Appella E, Blasi F (1986) Autocrine saturation of pro-**urokinase** receptors on human A431 cells. Cell 45: 675-684

DETD Stump D C, Lijnen H R, Collen D (1986a) Purification and characterization of single-chain **urokinase**-type plasminogen activator from human cell cultures. J Biol Chem 261: 1274-1278

DETD Stump D C, Thienpont M, Collen D (1986b) **Urokinase**-related proteins in human urine. J Biol Chem 261: 1267-1273

DETD Thorsen S, Glas-Greenwalt P, Astrup T (1972) Differences in the binding to fibrin of **urokinase** and tissue plasminogen activator. Thrombos Diathes Haemorrh 28: 65-74

DETD . . . T, de Serrano V S, Gaffney P J, Castellino F J (1988) The activation of human (Glu^1)plasminogen by human single-chain **urokinase**. Arch Biochem Biophys 264: 222-230

DETD . . . J-D, Baccino D, Belin D (1985) A cellular binding site for the M_r 55,000 form of the human plasminogen activator, **urokinase**. J Cell Biol 100: 86-92

DETD Wun T-C, Ossowski L, Reich E (1982) A proenzyme form of human **urokinase**. J Biol Chem 157: 7262-7268

CLM What is claimed is:

. . . a host cell, said vector comprising (a) a coding sequence which encodes a polypeptide, soluble in aqueous solution, and having **urokinase plasminogen activator** (**UPA**) binding activity, said polypeptide comprising a **UPA**-binding domain having an amino acid sequence which (i) is identical to SEQ ID NO:3, (ii) is identical to the amino acid sequence of a **UPA**-binding 16 kDa glycosylated chymotryptic fragment of the mature **uPA** receptor protein having the amino acid sequence shown in sequence (A), or (iii) differs in amino acid sequence from (i). . . operably linked to said coding sequence; with the proviso that said vector does not comprise any sequence encoding a mature **UPA** receptor having the amino acid sequence shown in Sequence A or its natural precursor.

2. The vector of claim 1 wherein the polypeptide comprises a **UPA**-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution. . .

3. The vector of claim 1 wherein the polypeptide comprises a

UPA-binding domain which is identical to SEQ ID NO:3.

. . . of claim 1 wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or differs therefrom only by a carboxy terminal truncation and/or by one or more. . .

. . . claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or differs therefrom solely by one or more conservative substitutions.

. . . claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

. . . claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A.

. . . polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the **UPA** receptor which has **UPA** binding activity, or to a sequence differing from that of said fragment by a single conservative substitution.

10. The vector of claim 1 wherein the polypeptide comprises said **uPA**-binding peptide moiety and a plasminogen activator inhibitor moiety.

11. The vector of claim 1 wherein the polypeptide comprises said **uPA**-binding peptide moiety and a heterologous peptide moiety, where the heterologous peptide moiety increases expression of said polypeptide in said host. . .

12. A method of producing a polypeptide which is soluble in aqueous solution and has **UPA** binding activity which method comprises (a) providing a host cell transformed with a compatible expression vector according to claim 1;. . .

13. The method of claim 12 wherein the polypeptide comprises a **UPA**-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution. . .

14. The method of claim 12, wherein the polypeptide comprises a **UPA**-binding domain which is identical to SEQ ID NO:3.

. . . of claim 12, wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or differs therefrom only by (A) a carboxy terminal truncation (B) one or more. . .

. . . claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or differs therefrom only by one or more conservative substitutions.

. . . claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

. . . claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human **UPA**-receptor having the sequence of Sequence A.

. . . polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the **UPA** receptor which has **UPA** binding activity, or to a sequence differing

from that of said fragment by a single conservative substitution.

21. The method of claim 12 wherein the polypeptide comprises said **uPA**-binding peptide moiety and a plasminogen activator inhibitor moiety.

22. The method of claim 12 wherein the polypeptide comprises said **uPA**-binding peptide moiety and a heterologous peptide moiety, where the heterologous peptide moiety increases expression of said polypeptide in said host. . .

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L14 ANSWER 4 OF 13 USPATFULL on STN
2001:93481 Method of treating a **urokinase**-type plasminogen activator-mediated disorder.
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Stratton-Thomas, Jennifer R., San Mateo, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 6248715 B1 20010619
APPLICATION: US 1995-438745 19950510 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
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=> d l14,cbib,kwic,4

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TI Method of treating a **urokinase**-type plasminogen activator-mediated disorder
AI US 1995-438745 19950510 (8) <--
AB A method of treating an **uPA**-mediated disorder is disclosed, which comprises providing and administering an effective amount of polypeptide consisting essentially of the EGF-like domain of human **uPA** or active analog thereof.
SUMM . . . relates to the fields of cellular biology and protein expression. More particularly, the invention relates to peptide ligands of the **urokinase plasminogen activator** receptor, and methods for preparing the same.
SUMM **Urokinase**-type plasminogen activator (**uPA**) is a multidomain serine protease, having a catalytic "B" chain (amino acids 144-411), and an **amino-terminal fragment** ("**ATF**", aa 1-143) consisting of a growth factor-like domain (4-43) and a kringle (aa 47-135). The **uPA** kringle appears to bind heparin, but not fibrin, lysine, or aminohex-anoic acid. The growth factor-like domain bears some similarity to. . . the structure of epidermal growth factor (EGF), and is thus also referred to as an "EGF-like" domain. The single chain pro-**uPA** is activated by plasmin, cleaving the chain into the two chain active form, which is linked together by a disulfide. . .
SUMM **uPA** binds to its specific cell surface receptor (uPAR). The binding interaction is apparently mediated by the EGF-like domain (S. A. Rabbani et al., J Biol Chem (1992) 267:14151-56). Cleavage of pro-**uPA** into active **uPA** is accelerated when pro-**uPA** and plasminogen are receptor-bound. Thus, plasmin activates pro-**uPA**, which in turn

activates more plasmin by cleaving plasminogen. This positive feedback cycle is apparently limited to the receptor-based proteolysis. . .

SUMM . . . extracellular proteolysis, fibrin clot lysis, tissue remodeling, developmental cell migration, inflammation, and metastasis. Accordingly, there is great interest in developing **uPA** inhibitors and **uPA** receptor antagonists. E. Appella et al., J Biol Chem (1987) 262:4437-40 determined that receptor binding activity is localized in the EGF-like domain, and that residues 12-32 appear to be critical for binding. The critical domain alone ($\mathbf{uPA}_{12-32}$) bound uPAR with an affinity of 40 nM (about 100 fold less than intact **ATF**).

SUMM . . . A. Rabbani et al., supra, disclosed that the EGF-like domain is fucosylated at Thr_{18}, and reported that fucosylated EGF-like domain ($\mathbf{uPA}_{4-43}$, produced by cleavage from pro-**uPA**) was mitogenic for an osteosarcoma cell line, SaOS-2. In contrast, non-fucosylated EGF-like domain bound uPAR with an affinity equal to. . .

SUMM One aspect of the invention is a method for producing non-fucosylated **uPA** EGF-like domain, particularly $\mathbf{uPA}_{1-48}$.

SUMM Another aspect of the invention is non-fucosylated $\mathbf{uPA}_{1-48}$, which is useful for inhibiting the mitogenic activity of **uPA** in cancer cells.

SUMM . . . aspect of the invention is a method for treating cancer and metastasis by administering an effective amount of a non-fucosylated **uPA** EGF-like domain, particularly $\mathbf{uPA}_{1-48}$.

SUMM Another aspect of the invention is a method treating a **uPA**-mediated disorder by administering a composition comprising an effective amount of a non-fucosylated polypeptide consisting of the EDF-like domain by instillation. . .

SUMM The term "huPA" refers specifically to human **urokinase**-type plasminogen activator. The "EGF-like domain" is that portion of the huPA molecule responsible for mediating huPA binding to its receptor. . .

SUMM . . . from other portions of the huPA protein. For example, a huPAR antagonist composition should contain less than 20 wt % **uPA** B domain (dry weight, absent excipients), preferably less than 10 wt % **uPA**-B, more preferably less than 5 wt % **uPA**-B, most preferably no detectable amount. The huPAR antagonist polypeptides also preferably exclude the kringle region of **uPA**.

SUMM The term "**uPA**-mediated disorder" refers to a disease state or malady which is caused or exacerbated by a biological activity of **uPA**. The primnary biological activity exhibited is plasminogen activation. Disorders mediated by plasminogen activation include, without limitation, inappropriate angiogenesis (e.g., diabetic. . . and the like), metastasis and invasion by tumor cells, and chronic inflammation (e.g., rheumatoid arthritis, emphysema, and the like). Fucosylaied **ATF** is also mitogenic for some tumor cells (e.g., SaOS-2 osteosar-coma cells), which sometimes self-activate in an autocrine mechanism. Accordingly, the huPAR antagonist of the invention is effective in inhibiting the proliferation of **uPA**-activated tumor cells.

SUMM The term "**pharmaceutically** acceptable" refers to compounds and compositions which may be administered to mammals without undue toxicity. Exemplary **pharmaceutically** acceptable salts include mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic. . .

SUMM . . . relies on the fact that yeast do not fucosylate proteins upon expression, but are able to express properly folded, active **uPA** and fragments. One may employ other eukaryotic hosts in the practice of the invention as long as the host is. . .

SUMM The expression vector is constructed according to known methods, and typically comprises a plasmid functional in the selected host. The **uPA** sequence used may be cloned following the method described in Example 1 below. Variations thereof (i.e., active fragments and active. . .

SUMM . . . assayed for activity by methods known in the art. For example, one may assay competition of the antagonist against native **uPA** for cell surface receptor binding. Competition for the receptor correlates

with inhibition of **uPA** biological activity. One may assay huPAR antagonist polypeptides for anti-mitogenic activity on appropriate tumor cell lines, such as the osteosarcoma. . .

SUMM . . . are administered orally, topically, or by parenteral means, including subcutaneous and intramuscular injection, implantation of sustained release depots, intravenous injection, **intranasal** administration, and the like. When used to treat tumors, it may be advantageous to apply the huPAR antagonist directly to. . . the site, e.g., during surgery to remove the bulk of the tumor. Accordingly, huPAR antagonist may be administered as a **pharmaceutical** composition comprising huPAR antagonist in combination with a **pharmaceutically** acceptable excipient. Such compositions may be aqueous solutions, emulsions, creams, ointments, suspensions, gels, liposomal suspensions, and the like. Suitable excipients. . . Pat. No. 5,124,155, incorporated herein by reference. Further, one may provide a huPAR antagonist in solid form, especially as a **lyophilized powder**. **Lyophilized** formulations typically contain stabilizing and bulking agents, for example human serum albumin, sucrose, mannitol, and the like. A thorough discussion of **pharmaceutically** acceptable excipients is available in Remington's **Pharmaceutical** Sciences (Mack Pub. Co.).

DETD DNA encoding residues 1-48 of mature human **uPA** (huPA) was cloned into a yeast expression vector as a fusion with the yeast alpha-factor leader (αF1), under transcriptional control. . .

DETD . . . the BamHI site, the cassette containing the ADH2-GAP hybrid promoter, the yeast ai-factor leader, the coding sequence for mature human **uPA**, and the GAP terminator, obtained from P. Valenzuela, Chiron Corporation) derived from a human kidney cDNA library (M. A. Truett. . .

DETD . . . eluted with a 0.6% gradient of acetonitrile containing 1% TFA. The major peak eluting at 33.5 minutes was collected and **lyophilized**. The purification yield is summarized in Table 1:

DETD . . . protein concentration due to interference with BCA assay

[b] Unit = volume of crude sample required to inhibit binding of ^{125}I-**ATF** 50% in competition with biotinylated suPAR.

DETD . . . of threonine was observed at cycle 18, indicating that this residue was not modified by fucosylation, as is found for **uPA** purified from eukaryotic cells. The absence of post translational modification was later confirmed by electrospray mass spectrometry. The binding activity. . .

DETD Baculovirus-derived recombinant human **urokinase** receptor was expressed as a truncated, soluble molecule as described previously for mouse L-cells (Masucci et al., J Biol Chem. . . purified receptor was biotinylated with NHS-biotin, and immobilized at 1 μg/mL in PBS/0.1% BSA on streptavidin coated 96-well plates. Human **uPA ATF** (residues 1-135, obtained from M. Shuman, University of California, San Francisco) was iodinated using the lodogen method (Pierce), and used as tracer. The ^{125}I-**ATF** was incubated at 100-500 pM with increasing amounts of $huPA_{1-48}$ in triplicate (100 pM-1 μM) for 2 hours at room. . . and the remaining bound radioactivity determined. The apparent K_d observed for $huPA_{1-48}$ was 0.3 nM, comparable to that reported for **ATF** and intact **uPA**.

DETD . . . the M13 pIII gene. The sequence of the insert is shown in SEQ ID NO:3. A synthetic gene encoding human **urokinase** residues 1-48 was obtained from J. Stratton-Thomas, Chiron Corporation.

DETD . . . transformed into E. coli JS5 (Biorad) by electroporation. Strain JS5 overproduces lac repressor, and is sup0, preventing expression of the $\mathbf{uPA_{1-48}}$ -pIII fusion protein due to the amber stop codon between the $\mathbf{uPA_{1-48}}$ and pIII genes. Correct clones were identified by restriction analysis and confirmed by DNA sequencing. These steps yielded phagemids pHM1a (M1Flag-$\mathbf{uPA_{1-48}}$) and pHM3a (Glutag-$\mathbf{uPA_{1-48}}$). The DNA sequences of the fusion proteins in

these phagemids are shown in SEQ ID NO: 10 and SEQ ID. . .

DETD The phagemid containing a synthetic gene for **uPA**$_{1-48}$ was constructed in the same vector by the following steps. The sequence of the synthetic gene is shown in SEQ. . . pCBRuPA (16 μg), a derivative of pCBR (Frederick et al., J Biol Chem (1990) 265:3793) containing this synthetic gene for **uPA**$_{1-48}$, inserted between the yeast α-factor leader and GAPDH terminator as a BglII fragment, was digested with Sac1 and Cla1, and. . .

DETD . . . then blocked with PBS containing 0.1% BSA. To the streptavidin plates was then added 1 μg/mL of biotinylated secreted human **urokinase** receptor obtained by recombinant baculovirus infection of A. californica Sf9 cells. After 2 hours at room temperature, the plates were. . .

DETD Table 2 shows that phagemids displaying **uPA**$_{1-48}$ are specifically bound and eluted from immobilized **urokinase** receptor. Table 3 demonstrates that the phagemid which displays a Glu tag-**uPA**$_{1-48}$ fusion is specifically retained by immobilized Glu Ab. Finally, Table 4 shows that a population of the Glu-**uPA**$_{1-48}$ phagemid which has been specifically eluted from the Glu Ab plates, is retained with a much higher yield on **urokinase** receptor plates, than is the unenriched phagemid population.

DETD These enriched phagemid pools are used for multiple mutagenesis strategies in order to identify improved **uPA**$_{1-48}$ ligands with altered specificity or improved affinity. For example the region between residues 13 and 32 of human **uPA** has been implicated in receptor binding (E. Appella et al., J Biol Chem (1987) 262:4437-40). Key residues in the region. . .

DETD In order to rapidly and quantitatively assess the binding affinities of the resulting **uPA**$_{1-48}$ variants, relatively large quantities of properly folded proteins are required. Although this could be done by bacterial expression, using the. . . To accomplish this we have constructed a yeast expression vector which enables us to move fragments encoding residues 4-48 of **uPA**$_{1-48}$ in a single step from the phagemid vectors. This was accomplished as follows: Plasmid pAGαG, identical to pCBR except for. . . treatment with alkaline phosphatase yielded a vector into which was ligated the BglII fragment corresponding to the synthetic gene for **uPA**$_{1-48}$. Transformation of E. coli strain HB101 to ampicillin resistance and restriction analysis yielded the correct clone. The 2.4 kB BamHI. . .

DETD Using this construct, one can express a library of **uPA** variations for screening. Variations may be constructed by a variety of methods, including low-fidelity PCR (which introduces a large number of random point mutations), site-specific mutation, primer-based mutagenesis, and ligation of the **uPA**$_{1-48}$ sequence (or portions thereof) to a random oligonucleotide sequence (e.g., by attaching $(NNS)_x$ to the **uPA**$_{1-48}$ coding sequence, or substituting NNS for one or more **uPA**$_{1-48}$ codons). Generation of random oligonucleotide sequences is detailed in Devlin, WO91/18980, incorporated herein by reference. Phage displaying **uPA**$_{1-48}$ variants (having one or more amino acid substitutions) are screened according to the protocol described above (using, e.g., pHM3a as. . .

DETD . . . site to be treated. The formulation is also generally suitable for administration as eyedrops directly to the conjunctiva, or by **intranasal** administration as an aerosol. Alternatively, a concentrated formulation (e.g., reducing the phosphate buffered saline to 2 mL) may be used. . .

CLM What is claimed is:

1. A method for treating a **urokinase**-type plasminogen activator (**uPA**)-mediated disorder, said method comprising: (i) providing a composition comprising a non-fucosylated polypeptide consisting

essentially of $huPA_{1-18}$ or an active peptide. . . binding affinity to huPAR as $huPA_{4-18}$, and (ii) administering an effective amount of said composition to a patient having a **uPA**-mediated disorder.

2. The method of claim 1, wherein said **uPA**-mediated disorder is selected from the group consisting of metastasis, inappropriate angiogenesis, and chronic inflammation.

3. The method of claim 1, wherein said **uPA**-mediated disorder is selected from the group consisting of Kaposi's sarcoma, diabetic retinopathy, and rheumatoid arthritis.

5. The method of claim 1, wherein said **uPA**-mediated disorder is metastasis.

6. The method of claim 1, wherein said **uPA**-mediated disorder is inappropriate angiogenesis.

8. The method of claim 1, wherein said **uPA**-mediated disorder is chronic inflammation.

9. The method of claim 1, wherein said **uPA**-mediated disorder is Kaposi's sarcoma.

10. The method of claim 1, wherein said **uPA**-mediated disorder is diabetic retinopathy.

11. The method of claim 1, wherein said **uPA**-mediated disorder is rheumatoid arthritis.

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     FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1          9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2           231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3           106 S L2 AND AD<MAR 01 2001
L4             8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)
L5            25 S L3 AND POWDER
L6            19 S L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L7             1 S L4 AND L6
L8             1 S L7 AND (POWDER)
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L10            1 S L9 AND PHARMACEUTICAL
L11            1 S L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)
L12           18 S L5 AND LYOPHILIZ?
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 16 Aug 2005 (20050816/PD)
FILE LAST UPDATED: 17 Aug 2005 (20050817/ED)
HIGHEST GRANTED PATENT NUMBER: US6931661
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HIGHEST APPLICATION PUBLICATION NUMBER: US2005177917
CA INDEXING IS CURRENT THROUGH 17 Aug 2005 (20050817/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 16 Aug 2005 (20050816/PD)
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FILE LAST UPDATED: 17 AUG 2005 (20050817/UP). FILE COVERS 1950 TO DATE.

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MeSH 2005 vocabulary.

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substance identification.

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FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1 9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2 231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3 106 S L2 AND AD<MAR 01 2001
L4 8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)

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L5           25 S L3 AND POWDER
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L8            1 S L7 AND (POWDER)
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L11           1 S L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)
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       974169 MOLECULAR
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        33953 PLASMINOGEN
        63440 ACTIVATOR
            5 HIGH MOLECULAR WEIGHT UROKINASE-TYPE PLASMINOGEN ACTIVATOR
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                ACTIVATOR)
         1316 HMW
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L17        109 L16 AND (1985-2001/PY)

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L17  ANSWER 1 OF 109     MEDLINE on STN
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2002057483. PubMed ID: 11783013. Experimental study of anti-metastasis effect of **urokinase amino-terminal fragment** gene on human breast cancer cells. Zhu F; Xing G; He F. (Beijing Institute of Radiation Medicine, Beijing 100850, China.) Zhonghua zhong liu za zhi [Chinese journal of oncology], **(2001 Mar)** 23 (2) 115-7. Journal code: 7910681. ISSN: 0253-3766. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To explore the suppressive effects of **urokinase amino-terminal fragment** (**ATF**) gene on metastatic potential of human breast cancer cell line MCF-7. METHODS: A pcDNA3-**ATF** plasmid containing **ATF** cDNA under CMV promotor/enhancer control was constructed and transfected into MCF-7 cells by lipofectin. The expression of of uPA/uPAR and **ATF** in MCF-7 cells were analyzed by RT-PCR and Western blot. The effect of **ATF** expression on invasiveness in vitro, tumorigenesis and metastasis in vivo of MCF-7 cell was investigated. RESULTS: MCF-7 cells displayed an overexpression of uPA/uPAR. Expression of **ATF** was detected after **ATF** gene-transfection. The invasive capacity of **ATF** gene-transfected MCF-7 cells was decreased significantly. Although the tumorigenesis was not affected, the in vivo metastasis of **ATF** gene-transfected MCF-7 cells was remarkably inhibited. CONCLUSION: Suppression of invasiveness and metastasis of **ATF**-transfected MCF-7 cells is perhaps due to a competitive inhibition of interaction with endogenous uPA/uPAR.

L17 ANSWER 2 OF 109 MEDLINE on STN

2001565487. PubMed ID: 11672584. **Urokinase**-type plasminogen activator up-regulates its own expression by endothelial cells and monocytes via the u-PAR pathway. Li C; Zhang J; Jiang Y; Gurewich V; Chen Y; Liu J N. (Institute of Molecular Medicine, Nanjing University, Nanjing 10008, China.) Thrombosis research, **(2001 Aug 1)** 103 (3) 221-32. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB Signal transduction by **urokinase**-type plasminogen activator (u-PA) bound to its cell receptor has been well established. In the present study, we found, for the first time to our knowledge, that u-PA promotes its own synthesis by endothelial cells and monocytes. This phenomenon was characterized and shown to involve the u-PA receptor (u-PAR) pathway. The finding may be of general importance, since most cells that express u-PAR also produce u-PA. Human umbilical vein endothelial cells (HUVECs), U937 monocytes, and human peripheral blood monocytes (PFMCs) were incubated with diisopropylfluorophosphate (DFP)-pretreated u-PA, the **amino-terminal fragment** (**ATF**) of u-PA, or the kringle domain. A threefold up-regulation of u-PA secretion and synthesis by u-PA or **ATF** was found. The predominant effect was expressed in HUVECs, in which u-PA mRNA was also up-regulated. The u-PA kringle domain had no effect on u-PA synthesis, leading to the conclusion that the EGF domain was responsible. This was also consistent with the additional finding that the u-PAR, to which the EGF domain binds, was necessary for the up-regulation. The results indicate that u-PA up-regulates itself via its EGF domain and u-PAR. The possibilities that the results were related to displacement of receptor-bound u-PA or the blocking of u-PA incorporation into the cells were excluded. A modest up-regulation of u-PAR was also associated with this phenomenon.

L17 ANSWER 3 OF 109 MEDLINE on STN

2001545564. PubMed ID: 11592401. Cyclo19,31[D-Cys19]-uPA19-31 is a potent competitive antagonist of the interaction of **urokinase**-type plasminogen activator with its receptor (CD87). Magdolen V; Burgle M; de Prada N A; Schmiedeberg N; Riemer C; Schroeck F; Kellermann J; Degitz K; Wilhelm O G; Schmitt M; Kessler H. (Frauenklinik der Technischen Universitat Munchen, Germany.) Biological chemistry, **(2001 Aug)** 382 (8) 1197-205. Journal code: 9700112. ISSN: 1431-6730. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) represents a central molecule

in pericellular proteolysis and is implicated in a variety of physiological and pathophysiological processes such as tissue remodelling, wound healing, tumor invasion, and metastasis. uPA binds with high affinity to a specific cell surface receptor, uPAR (CD87), via a well defined sequence within the N-terminal region of uPA (uPA19-31). This interaction directs the proteolytic activity of uPA to the cell surface which represents an important step in tumor cell proliferation, invasion, and metastasis. Due to its fundamental role in these processes, the uPA/uPAR-system has emerged as a novel target for tumor therapy. Previously, we have identified a synthetic, cyclic, uPA-derived peptide, cyclo19,31uPA19-31, as a lead structure for the development of low molecular weight uPA-analogues, capable of blocking uPA/uPAR-interaction [Burgle et al., Biol. Chem. 378 (1997), 231-237]. We now searched for peptide variants of cyclo19,31uPA19-31 with elevated affinities for uPAR binding. Among other tasks, we performed a systematic D-amino acid scan of uPA19-31, in which each of the 13 L-amino acids was individually substituted by the corresponding D-amino acid. This led to the identification of cyclo19,31[D-Cys19]-uPA19-31 as a potent inhibitor of uPA/uPAR-interaction, displaying only a 20 to 40-fold lower binding capacity as compared to the naturally occurring uPAR-ligands uPA and its **amino-terminal fragment**. Cyclo19,31[D-Cys19]-uPA19-31 not only blocks binding of uPA to uPAR but is also capable of efficiently displacing uPAR-bound uPA from the cell surface and to inhibit uPA-mediated, tumor cell-associated plasminogen activation and fibrin degradation. Thus, cyclo19,31[D-Cys19]-uPA19-31 represents a promising therapeutic agent to significantly affect the tumor-associated uPA/uPAR-system.

L17 ANSWER 4 OF 109 MEDLINE on STN

2001346125. PubMed ID: 11410166. cDNA transfection of **amino-terminal fragment** of **urokinase** efficiently inhibits cancer cell invasion and metastasis. Zhu F; Jia S; Xing G; Gao L; Zhang L; He F. (Beijing Institute of Radiation Medicine, Beijing, China.) DNA and cell biology, **(2001 May)** 20 (5) 297-305. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB Focusing of **urokinase**-type plasminogen activator (uPA) to the cell surface via binding to its specific receptor (uPAR, CD87) is critical for tumor invasion and metastasis. Consequently, the inhibition of uPA-uPAR interaction on the cell surface might be a promising anti-invasion and anti-metastasis strategy. We examined the effects of cDNA transfection of the human uPA **amino-terminal fragment** (**ATF**) on invasion and metastasis of cancer cells. First, a highly metastatic human lung giant-cell carcinoma cell line (PG), used as the target cell for evaluation of this effect, was demonstrated to express both uPA and uPAR. Then, **ATF**, which contains an intact uPAR binding site but is catalytically inactive, was designed as an antagonist of uPA-uPAR interaction and was transfected into PG cells. [(3)H]-Thymidine incorporation and cell growth curves indicated that expressed **ATF** did not affect the proliferation of transfected cells. However, analysis by scanning electron microscopy revealed that **ATF** changed the host cells from the typical invasive phenotype to a noninvasive one. Correspondingly, the modified Boyden chamber test in vitro showed that **ATF** expression significantly decreased the invasive capacity of transfected cells. Furthermore, in the spontaneous metastasis model, it was confirmed in vivo that expressed **ATF** remarkably inhibited lung metastasis of implanted **ATF**-transfected PG cells. In summary, autocrine **ATF** could act as an antagonist of uPA-uPAR interaction, and **ATF** cDNA transfection could efficiently inhibit the invasion and metastasis of the cancer cells. Inhibition of uPA-uPAR interaction on the cell surface might be a promising anti-invasion and anti-metastasis strategy.

L17 ANSWER 5 OF 109 MEDLINE on STN

2001327647. PubMed ID: 11394884. **Amino-terminal fragment** of

urokinase-type plasminogen activator inhibits HIV-1 replication. Wada M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10 Murotani, Nishi-ku, Kobe, 651-2241, Japan.. wada-m@jcrpharm.co.jp) . Biochemical and biophysical research communications, **(2001 Jun 8)** 284 (2) 346-51. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB CD8+ T lymphocytes have been shown to produce unidentified soluble factors active in suppressing HIV-1 replication. In this study, we purified an HIV-1 suppressing activity from the culture supernatant of an immortalized CD8+ T cell clone, derived from an HIV-1 infected long-term nonprogressor, and identified this activity as the **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA). **ATF** is catalytically inactive, but suppresses the release of viral particles from the HIV-1 infected cell lines via binding to its receptor CD87. In contrast, cell proliferation and the secretion of an HIV-1 LTR driven reporter gene product were not affected by **ATF**. These findings suggest that **ATF** may inhibit the assembly and budding of HIV-1, which provides a novel therapeutic strategy for AIDS.

L17 ANSWER 6 OF 109 MEDLINE on STN

2001324898. PubMed ID: 11319620. In vivo suppression of restenosis in balloon-injured rat carotid artery by adenovirus-mediated gene transfer of the cell surface-directed plasmin inhibitor **ATF**.BPTI. Lamfers M L; Lardenoye J H; de Vries M R; Aalders M C; Engelse M A; Grimbergen J M; van Hinsbergh V W; Quax P H. (Gaubius Laboratory TNO-PG, 2301 CE Leiden, The Netherlands.) Gene therapy, **(2001 Apr)** 8 (7) 534-41. Journal code: 9421525. ISSN: 0969-7128. Pub. country: England: United Kingdom. Language: English.

AB Injury-induced neointimal development results from migration and proliferation of vascular smooth muscle cells (SMC). Cell migration requires controlled proteolytic degradation of extracellular matrix surrounding the cell. Plasmin is a major contributor to this process by degrading various matrix proteins directly, or indirectly by activating matrix metalloproteinases. This makes it an attractive target for inhibition by gene transfer. An adenoviral vector, Ad.**ATF**.BPTI, was constructed encoding a hybrid protein, which consists of the aminoterminal fragment (**ATF**) of **urokinase**-type plasminogen activator (u-PA) linked to bovine pancreas trypsin inhibitor (BPTI), a potent inhibitor of plasmin. This hybrid protein binds to the u-PA receptor, thereby inhibiting plasmin activity at the cell surface, and was found to be a potent inhibitor of cell migration in vitro. Local infection with Ad.**ATF**.BPTI of balloon-injured rat carotid artery resulted in detectable expression of **ATF**.BPTI mRNA and protein in the vessel wall. Morphometric analysis of arterial cross-sections revealed that delivery of Ad.**ATF**.BPTI to the carotid artery wall at the time of balloon injury inhibited neointima formation by 53% ($P < 0.01$) at 14 days and 19% (P = NS) at 28 days after injury when compared with control vector-infected arteries. Intima/media ratios were decreased by 60% ($P < 0.01$) and 35% ($P < 0.05$) at 14 and 28 days, respectively, when compared with control vector-infected arteries. Furthermore, a small but significant increase in medial area was found in the Ad.**ATF**.BPTI-treated arteries at 28 days ($P < 0.05$). These results show that local infection of the vessel wall with Ad.**ATF**.BPTI reduces neointima formation, presumably by inhibiting SMC migration, thereby offering a novel therapeutic approach to inhibiting neointima development.

L17 ANSWER 7 OF 109 MEDLINE on STN

2001226300. PubMed ID: 11157723. Adenoviral expression of a **urokinase** receptor-targeted protease inhibitor inhibits neointima formation in murine and human blood vessels. Quax P H; Lamfers M L; Lardenoye J H; Grimbergen J M; de Vries M R; Slomp J; de Ruiter M C; Kockx M M; Verheijen

J H; van Hinsbergh V W. (Gaubius Laboratory TNO-PG, Leiden, Netherlands.. pha.quax@pg.tno.nl) . Circulation, **(2001 Jan 30)** 103 (4) 562-9. Journal code: 0147763. ISSN: 1524-4539. Pub. country: United States. Language: English.

AB BACKGROUND: Smooth muscle cell migration, in addition to proliferation, contributes to a large extent to the neointima formed in humans after balloon angioplasty or bypass surgery. Plasminogen activator/plasmin-mediated proteolysis is an important mediator of this smooth muscle cell migration. Here, we report the construction of a novel hybrid protein designed to inhibit the activity of cell surface-bound plasmin, which cannot be inhibited by its natural inhibitors, such as alpha(2)-antiplasmin. This hybrid protein, consisting of the receptor-binding **amino-terminal fragment** of uPA (**ATF**), linked to the potent protease inhibitor bovine pancreas trypsin inhibitor (BPTI), can inhibit plasmin activity at the cell surface. METHODS AND RESULTS: The effect of adenovirus-mediated **ATF**.BPTI expression on neointima formation was tested in human saphenous vein organ cultures. Infection of human saphenous vein segments with Ad.CMV.**ATF**.BPTI (5x10(9) pfu/mL) resulted in 87.5+/-3.8% (mean+/-SEM, n=10) inhibition of neointima formation after 5 weeks, whereas Ad.CMV.**ATF** or Ad.CMV.BPTI virus had only minimal or no effect on neointima formation. The efficacy of **ATF**.BPTI in vivo was demonstrated in a murine model for neointima formation. Neointima formation in the femoral artery of mice, induced by placement of a polyethylene cuff, was strongly inhibited (93.9+/-2%) after infection with Ad.CMV.mATF.BPTI, a variant of **ATF**.BPTI able to bind specifically to murine uPA receptor; Ad.CMV.mATF and Ad.CMV.BPTI had no significant effect. CONCLUSIONS: These data provide evidence that adenoviral transfer of a hybrid protein that binds selectively to the uPA receptor and inhibits plasmin activity directly on the cell surface is a powerful approach to inhibiting neointima formation and restenosis.

L17 ANSWER 8 OF 109 MEDLINE on STN

2001126569. PubMed ID: 11206835. Isolation and characterization of cell lines with reduced **urokinase** binding. Lau H K; Teitel J M; Kim M. (Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital and University of Toronto, Ontario, Canada.. lauh@smh.toronto.on.ca) . Clinical & experimental metastasis, **(2000)** 18 (1) 29-36. Journal code: 8409970. ISSN: 0262-0898. Pub. country: Netherlands. Language: English.

AB Six cell lines have been generated from the human fibrosarcoma HT-1080 by mutagenesis. They were selected on the basis of reduced **urokinase** (uPA) binding on replicate polyester filters. Single cell clones were then isolated by limited dilution cloning. All cloned cells showed less uPA binding on filters, and as cell monolayers. These cell lines were able to bind only 10 to 65% as much uPA as the wild-type HT-1080 cells. Surface-bound uPA proteolytic activity and surface activation of plasminogen from these cells were also reduced relative to the wild-type. uPA could activate MAP kinases in the wild-type and two of the cell lines with the least uPA-binding, but the amount of the activated forms of the signalling molecules were reduced. Immunoblotting using two different anti-uPA receptor antibodies showed two cross-reacting protein species of approximately 53 kDa and approximately 38 kDa. The proportion of the lower Mr band to the higher Mr band was found to be reduced in all the cell lines relative to the wild-type. Chemical cross-linking with single-chain **urokinase** (scuPA) showed only one high-molecular-weight adduct, with Mr approximately 90 kDa, in all the cell lines tested. Similarly, cross-linking with the **amino terminal fragment** of uPA yielded a single approximately 70 kDa adduct. These would indicate that only the approximately 53 kDa band was responsible for cross-linking reactions. Equilibrium binding experiments showed that only one set of high-affinity binding sites for the wild-type cells. However, the binding of scuPA to two of these cell lines was best fitted to a two-site model, one of which was similar to the high-affinity binding sites of the

wild-type, although the number of sites was reduced, while the other was of much lower affinity but was large in number. These results are discussed in relation to changes in the structure of ligand binding machinery in these cells, which affect other cellular functions.

L17 ANSWER 9 OF 109 MEDLINE on STN

2000424232. PubMed ID: 10943860. Cartilage degradation and invasion by rheumatoid synovial fibroblasts is inhibited by gene transfer of a cell surface-targeted plasmin inhibitor. van der Laan W H; Pap T; Ronday H K; Grimbergen J M; Huisman L G; TeKoppele J M; Breedveld F C; Gay R E; Gay S; Huizinga T W; Verheijen J H; Quax P H. (Gaubius Laboratory, The Netherlands Organization for Applied Scientific Research, Prevention and Health, and Leiden University Medical Center.) Arthritis and rheumatism, **(2000 Aug)** 43 (8) 1710-8. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE: Joint destruction in rheumatoid arthritis (RA) is a result of degradation and invasion of the articular cartilage by the pannus tissue. The present study was undertaken to examine the role of the plasminogen activation system in cartilage degradation and invasion by synovial fibroblasts and investigate a novel gene therapeutic approach using a cell surface-targeted plasmin inhibitor (**ATF**.BPTI). METHODS: Adenoviral vectors were used for gene transfer. The effects of **ATF**.BPTI gene transfer on RA synovial fibroblast-dependent cartilage degradation were studied in vitro, and cartilage invasion was studied in vivo in the SCID mouse coimplantation model. RESULTS: The results indicate that cartilage matrix degradation by rheumatoid synovial fibroblasts is plasmin mediated and depends on **urokinase**-type plasminogen activator for activation. Targeting plasmin inhibition to the cell surface of the fibroblasts by gene transfer of a cell surface-binding plasmin inhibitor resulted in a significant reduction of cartilage matrix degradation in vitro and of cartilage invasion in vivo. Compared with uninfected rheumatoid synovial fibroblasts, the mean +/-SEM cartilage degradation in vitro was reduced to 87.9+/-0.9% after LacZ gene transfer versus a reduction to 24.0+/-1.6% after **ATF**.BPTI gene transfer ($P<0.0001$). The mean +/- SEM in vivo cartilage invasion score was 3.1+/-0.4 in the control-transduced fibroblasts and 1.8+/-0.4 in the **ATF**.BPTI-transduced fibroblasts ($P<0.05$). CONCLUSION: These results indicate a role of the plasminogen activation system in synovial fibroblast-dependent cartilage degradation and invasion in RA, and demonstrate an effective way to inhibit this by gene transfer of a cell surface-targeted plasmin inhibitor.

L17 ANSWER 10 OF 109 MEDLINE on STN

2000179850. PubMed ID: 10713063. Recombinant toxins that bind to the **urokinase** receptor are cytotoxic without requiring binding to the alpha(2)-macroglobulin receptor. Rajagopal V; Kreitman R J. (Laboratory of Molecular Biology, Division of Basic Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892, USA.) Journal of biological chemistry, **(2000 Mar 17)** 275 (11) 7566-73. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The alpha(2-)macroglobulin receptor (alpha(2)MR) has been reported to mediate the internalization of the **urokinase** plasminogen activator receptor (uPAR) via ligand binding to both receptors. To target malignant uPAR-expressing cells and to determine whether uPAR can internalize without ligand binding to alpha(2)MR, we engineered two recombinant toxins, **ATF**-PE38 and **ATF**-PE38KDEL. Each consists of the **amino-terminal fragment** (**ATF**) of human **urokinase** and a truncated form of Pseudomonas exotoxin (PE) devoid of domain Ia, which binds alpha(2)MR. **ATF**-PE38 and **ATF**-PE38KDEL were cytotoxic toward malignant uPAR-bearing cells, with IC(50) values as low as 0.02 ng/ml (0.3 pM). Cytotoxicity could be blocked using either recombinant **urokinase** or free **ATF**, indicating that the cytotoxicity of the recombinant toxins was specific. Radiolabeled **ATF**-PE38 had high affinity for uPAR (K(d) = 0.4-8 nM) on a variety of different malignant cell types and internalized

at a rate similar to that of **ATF**. The cytotoxicity was not diminished by receptor-associated protein, which binds and shields the alpha(2)MR from other proteins, or by incubation with phorbol myristate acetate, which is known to decrease the number of alpha(2)MRs in U937 cells or by antibodies to alpha(2)MR. Therefore, these recombinant toxins appear to internalize via uPAR without association with the alpha(2)MR.

L17 ANSWER 11 OF 109 MEDLINE on STN

2000125944. PubMed ID: 10657995. Cytosolic immunization allows the expression of preATF-saporin chimeric toxin in eukaryotic cells. Fabbrini M S; Carpani D; Soria M R; Ceriotti A. (Department of Biological and Technological Research-Dibit, San Raffaele Scientific Institute, 20132 Milano, Italy.. fabbrini.serena@hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, **(2000 Feb)** 14 (2) 391-8. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB In this work, we have devised an intracellular immunization strategy for the expression in high amounts of **ATF**-saporin, a targeted chimeric toxin constituted by the **ATF** receptor binding domain of human **urokinase** and the plant ribosome-inactivating protein saporin, which has been shown to be highly cytotoxic to target cells. This strategy may allow the production of highly toxic secretory proteins in eukaryotic cells, avoiding cell suicide caused by autointoxication. The procedure consists of equipping host cells with cytosolic neutralizing antibodies directed toward the toxic domain of the heterologous polypeptide. We show that this intracellular immunization is essential for the synthesis of correctly folded, biologically active **ATF**-SAP in the high amounts needed to investigate its in vivo anti-metastatic potential. Such a strategy should be generally useful for the production of toxic molecules of therapeutic value whose folding and maturation require transit through the eukaryotic secretory pathway. Fabbrini, M. S., Carpani, D., Soria, M. R., Ceriotti, A. Cytosolic immunization allows the expression of preATF-saporin chimeric toxin in eukaryotic cells.

L17 ANSWER 12 OF 109 MEDLINE on STN

2000102888. PubMed ID: 10634825. Lysophosphatidylcholine induces **urokinase**-type plasminogen activator and its receptor in human macrophages partly through redox-sensitive pathway. Oka H; Kugiyama K; Doi H; Matsumura T; Shibata H; Miles L A; Sugiyama S; Yasue H. (Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto City, Japan.) Arteriosclerosis, thrombosis, and vascular biology, **(2000 Jan)** 20 (1) 244-50. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) and its cell surface receptor (uPAR) have been shown to be expressed in macrophages in atherosclerotic arterial walls, but the regulatory mechanisms of their expression remain unclear. The present study was performed to examine the effects of lysophosphatidylcholine (lysoPC), an important atherogenic lipid, on the expression of uPA and uPAR in human monocyte-derived macrophages. LysoPC upregulated the mRNA expression of uPA and uPAR, and it increased the protein expression of uPA in the culture medium and bound to the cell surface and of uPAR in the particulate fraction of the cells. LysoPC significantly increased the binding of the **amino-terminal fragment** of uPA to the treated cells and the cell-associated plasminogen activator activity. LysoPC stimulated superoxide anion production and increased intracellular oxidant levels in the cells. The combined incubation with reduced glutathione diethyl ester or N-acetylcysteine, antioxidants, suppressed the upregulation of uPA and uPAR mRNA and the increase in plasminogen activator activity by lysoPC. uPA and uPAR mRNA expression was also induced by the incubation with xanthine and xanthine oxidase, a superoxide anion-generating system. The results suggest that lysoPC increased the expression of uPA and uPAR and their functional activities in human monocyte-derived macrophages, at least in part through a

redox-sensitive mechanism. This coordinate increase in the expression of uPA and uPAR in human macrophages by lysoPC could play an important role in plaque formation and disruption, arterial remodeling, and angiogenesis in atherosclerotic arterial walls.

L17 ANSWER 13 OF 109 MEDLINE on STN
2000075754. PubMed ID: 10609663. Systemic delivery of antiangiogenic adenovirus AdmATF induces liver resistance to metastasis and prolongs survival of mice. Li H; Griscelli F; Lindenmeyer F; Opolon P; Sun L Q; Connault E; Soria J; Soria C; Perricaudet M; Yeh P; Lu H. (CNRS UMR 1582/Rhone-Poulenc Rorer Gencell, Institut Gustave Roussy, Villejuif, France.) Human gene therapy, **(1999 Dec 10)** 10 (18) 3045-53. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Systemic administration of Ad5-based recombinant adenovirus leads to preferential transduction of the liver. Using this property, we have assessed the potential of venous viral injection to deliver a recombinant antiangiogenic adenovirus to treat cancer dissemination and improve survival. The results demonstrate that venous injection of adenovirus AdmATF, which encodes a secretable mouse **ATF** (**amino-terminal fragment** of **urokinase**) known to inhibit angiogenesis, suppressed angiogenesis induced by colon cancer metastasis growth in mice liver and improved survival. Nude mice were injected intravenously with 5 X 10(9) PFU of AdmATF and subsequently challenged after a 3-day interval by intrasplenically injected human colon carcinoma cells (LS174T, 3 x 10(6)) that home to liver. Microscopic inspection revealed that, within the AdmATF-pretreated mice (n = 8), the size and number of liver-metastasized nodules on day 30 were remarkably reduced (80% in number, $p < 0.05$) compared with control mice (n = 7) pretreated in parallel with a control adenovirus. Metastatic growth-related liver weight gain was also inhibited up to 90%. AdmATF-specific capability that offers liver resistance to the apparition and growth of liver metastasis was shown to correlate with the inhibition of peritumoral and intratumoral angiogenesis (reduced by 79%, $p < 0.01$ as shown by anti-vWF immunostaining of liver sections) and a twofold increase in tumor necrotic area and an eightfold increase in apoptotic tumor cell number. This protective effect was still observed when the mice were challenged 10 days after venous AdmATF injection (visible metastasis nodules: 6.3+/-3.1, n = 7 for control mice versus 2.7+/-2.9, n = 10 for treated mice, $p < 0.05$). More importantly, the mean survival has been prolonged from 45.1 days (n = 9) to 83.3 days (n = 10, $p < 0.05$). Altogether, the high efficacy, although transient, in this experimental mice model strongly advocates the plausibility of transforming the liver into a dissemination resistant organ by antiangiogenic gene therapy through systemic delivery approach.

L17 ANSWER 14 OF 109 MEDLINE on STN
2000033582. PubMed ID: 10564640. In vivo angiogenic activity of **urokinase**: role of endogenous fibroblast growth factor-2. Ribatti D; Leali D; Vacca A; Giuliani R; Gualandris A; Roncali L; Nolli M L; Presta M. (Institute of Human Anatomy, University of Bari, Piazza G. Cesare 11, Italy.) Journal of cell science, **(1999 Dec)** 112 (Pt 23) 4213-21. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In vitro experimental evidences suggest that the proteolytic degradation of the extracellular matrix (ECM) by activation of the **urokinase**-type plasminogen activator (uPA)/plasmin system may affect growth factor activity and bioavailability. However, no direct in vivo observations were available to support this hypothesis. Here we demonstrate that endothelial GM 7373 cells overexpressing human uPA (uPA-R5 cells) cause the release of (125)I-labeled fibroblast growth factor-2 (FGF2) from endothelial ECM in a plasmin-dependent manner. Accordingly, uPA-R5 cells are angiogenic in vivo when applied on the top of the chorioallantoic membrane (CAM) of the chick embryo. In contrast, mock-transfected Neo2

cells are unable to release ECM-bound (125)I-FGF2 and are poorly angiogenic. Neovascularization elicited by uPA-R5 cells is significantly reduced by neutralizing anti-FGF2 antibodies to values similar to those observed in Neo2 cell-treated CAMs. Accordingly, purified human uPA stimulates neovascularization of the CAM in the absence of an inflammatory response. The angiogenic activity of uPA is significantly inhibited by neutralizing anti-FGF2 antibodies or by pretreatment with phenylmethylsulfonyl fluoride. The non-catalytic, receptor-binding **amino-terminal fragment** of uPA is instead non angiogenic. Taken together, the data indicate that uPA is able to induce angiogenesis in vivo via a plasmin-dependent degradation of ECM that causes the mobilization of stored endogenous FGF2.

L17 ANSWER 15 OF 109 MEDLINE on STN

2000005706. PubMed ID: 10537314. **Urokinase** receptor interacts with alpha(v)beta5 vitronectin receptor, promoting **urokinase**-dependent cell migration in breast cancer. Carriero M V; Del Vecchio S; Capozzoli M; Franco P; Fontana L; Zannetti A; Botti G; D'Aiuto G; Salvatore M; Stoppelli M P. (National Cancer Institute, Naples, Italy.. stoppelli@iigbna.iigb.na.cnr.it) . Cancer research, **(1999 Oct 15)** 59 (20) 5307-14. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Perturbation of adhesive interactions at cell-substratum and cell-cell contact sites is a critical event in the multistep process of cancer invasion. Recent studies indicate that the **urokinase** receptor (uPAR) is associated in large molecular complexes with other molecules, such as integrins. To test the possibility that uPAR may physically and functionally interact with vitronectin (Vn) receptors, we determined the expression level of uPAR, alpha(v)beta3, and alpha(v)beta5 Vn receptors in 10 human breast carcinomas. Here, we show the ability of uPAR to physically associate with alpha(v)beta5 in the breast carcinomas examined. The functional effects of this interaction were studied using HT1080 human fibrosarcoma and MCF-7 human breast carcinoma cell lines, both exhibiting a **urokinase**-dependent physical association between uPAR and alpha(v)beta5. Both cell lines respond to **urokinase** or to its noncatalytic **amino-terminal fragment** by exhibiting remarkable cytoskeletal rearrangements that are mediated by alpha(v)beta5 and require protein kinase C activity. On the contrary, binding of Vn to alpha(v)beta5 results in the protein kinase C-independent formation of F-actin containing microspike-type structures. Furthermore, alpha(v)beta5 is required for **urokinase**-directed, receptor-dependent MCF-7 and HT1080 cell migration. These data show that uPAR association with alpha(v)beta5 leads to a functional interaction of these receptors and suggest that uPAR directs cytoskeletal rearrangements and cell migration by altering alpha(v)beta5 signaling specificity.

L17 ANSWER 16 OF 109 MEDLINE on STN

1999384291. PubMed ID: 10454570. Role of distinct mitogen-activated protein kinase pathways and cooperation between Ets-2, **ATF**-2, and Jun family members in human **urokinase**-type plasminogen activator gene induction by interleukin-1 and tetradecanoyl phorbol acetate. Cirillo G; Casalino L; Vallone D; Caracciolo A; De Cesare D; Verde P. (International Institute of Genetics and Biophysics, CNR, 80125 Naples, Italy.) Molecular and cellular biology, **(1999 Sep)** 19 (9) 6240-52. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB We have investigated the in vivo and in vitro regulation of the human **urokinase**-type plasminogen activator (uPA) gene by interleukin-1 (IL-1) and analyzed the transcription factors and signalling pathways involved in the response of the -2.0-kb uPA enhancer to IL-1 induction and to tetradecanoyl phorbol acetate (TPA) induction. Mutational analysis showed the cooperative activity of the Ets-binding site (EBS) and the two AP-1 elements of the enhancer. The results reveal that the EBS is required for

the response to both inducers mediated by Ets-2, which is regulated at a level subsequent to DNA binding, by an IL-1- and phorbol ester-inducible transactivation domain. Both the IL-1 and the TPA-mediated induction result in a drastic increase of AP-1 binding to the downstream site of the enhancer (uPA 3' TPA-responsive element), while a mostly qualitative change, resulting from the interplay between **ATF**-2 homodimers and c-Jun-**ATF**-2 heterodimers, takes place at the upstream AP-1 element. The analysis of two distinct mitogen-activated protein kinase pathways shows that stress-activated protein kinase-Jun N-terminal kinase activation, resulting in the phosphorylation of **ATF**-2, c-Jun, and JunD, is required not only for the IL-1- but also for the TPA-dependent induction, while the extracellular signal-related kinase 1 (ERK-1) and ERK-2 activation is involved in the TPA- but not in the IL-1-dependent stimulation of the uPA enhancer.

L17 ANSWER 17 OF 109 MEDLINE on STN

1999318816. PubMed ID: 10388537. **Urokinase**-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. Yebra M; Goretzki L; Pfeifer M; Mueller B M. (Department of Immunology, The Scripps Research Institute, 10550 North Torrey Pines Road, IMM13, La Jolla, California, 92037, USA.) Experimental cell research, **(1999 Jul 10)** 250 (1) 231-40. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) participate in matrix degradation and cell migration by focusing proteolysis and functioning as a signaling ligand/receptor complex. uPAR, anchored by a lipid moiety in the membrane, is thought to require a transmembrane adapter to transduce signals into the cytoplasm. To study uPAR signaling, we transfected the prostate carcinoma cell line LNCaP, which does not express endogenous uPA or uPAR, with a uPAR encoding cDNA, resulting in high-level surface expression. We studied migration of these cells on fibronectin, which is mediated by the integrin alpha5beta1. Ligation of uPAR with uPA or its **amino-terminal fragment** enhanced haptotactic migration to fibronectin. In cells on fibronectin, but not on poly-l-lysine, ligation of uPAR also resulted in tyrosine phosphorylation of several proteins, including two proteins involved in integrin signaling, focal adhesion kinase and the crk-associated substrate p130(Cas). Furthermore, after uPAR ligation, uPAR was co-immunoprecipitated with beta1 integrins from the detergent-insoluble fraction of cell lysates. Thus, our data suggest that uPAR occupancy results in an interaction between uPAR and integrins and a potentiation of integrin-mediated signaling, which leads to enhanced cell migration. Copyright 1999 Academic Press.

L17 ANSWER 18 OF 109 MEDLINE on STN

1999293019. PubMed ID: 10362798. Role and localization of **urokinase** receptor in the formation of new microvascular structures in fibrin matrices. Kroon M E; Koolwijk P; van Goor H; Weidle U H; Collen A; van der Pluijm G; van Hinsbergh V W. (Gaubius Laboratory, Leiden University Hospital, Groningen Leiden The Netherlands.) American journal of pathology, **(1999 Jun)** 154 (6) 1731-42. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Fibrin or a fibrinous exudate can facilitate angiogenesis in many pathological conditions. In vitro, the outgrowth of capillary-like structures in fibrin can be mimicked by exposing human microvascular endothelial cells (hMVECs) to an angiogenic growth factor and tumor necrosis factor (TNF)-alpha. **Urokinase**-type plasminogen activator (u-PA) and plasmin activities are required for this angiogenic process. This study focuses on the role and localization of the u-PA receptor (u-PAR) in newly formed microvascular structures. The u-PAR-blocking monoclonal antibody (MAb) H-2 completely inhibited the formation of capillary-like tubular structures induced by exposure of hMVECs to basic fibroblast growth factor and TNF-alpha. This was accompanied by a

several-fold increase in u-PA accumulation in the conditioned medium. The effect of MAb H-2 was not caused by blocking cellular activation by u-PA/u-PAR interaction, as the **amino-terminal fragment** (**ATF**) of u-PA, which also activates u-PAR, prevented tube formation. In addition, the inhibition by MAb H-2 was not due to an effect of the antibody on u-PAR-vitronectin binding. These data show that inhibition of tube formation can be caused not only by inhibition of u-PA or plasmin activities but also by unavailability of the u-PAR for cell-bound proteolysis. Immunohistochemical analysis showed that in in vitro angiogenesis u-PAR and u-PA were localized on the invading, tube-forming hMVECs and not on the endothelial cells that are located on top of the fibrin matrix. u-PAR and u-PA were also prominently expressed on endothelial cells of neovessels present in an atherosclerotic plaque. These data may give more insight into the role of u-PAR in repair-associated angiogenesis.

L17 ANSWER 19 OF 109 MEDLINE on STN

1999272427. PubMed ID: 10339491. Characterization of cell-associated plasminogen activation catalyzed by **urokinase**-type plasminogen activator, but independent of **urokinase** receptor (uPAR, CD87). Longstaff C; Merton R E; Fabregas P; Felez J. (The National Institute for Biological Standards and Control, South Mimms, Hertfordshire, UK.. clongstaff@nibsc.ac.uk) . Blood, **(1999 Jun 1)** 93 (11) 3839-46. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB The 55-kD **urokinase** (uPA) receptor (uPAR, CD87) is capable of binding uPA and may be involved in regulating cell-associated plasminogen activation and pericellular proteolysis. While investigating the relationship between uPAR levels and plasmin generation, we found that uPA-catalyzed plasminogen activation is stimulated by cells which do not express uPAR. This uPAR-independent mechanism appears to be at least as effective in vitro as uPAR-dependent stimulation, such that stimulation on the order of 30-fold was observed, resulting from improvements in both apparent kcat and apparent Km. The mechanism depends on simultaneous binding of both uPA and plasminogen to the cell and requires the presence of the **amino-terminal fragment** (**ATF**), available in single chain and two chain high-molecular-weight uPA, but not low-molecular-weight uPA. Stimulation was observed in all leukemic cell lines investigated at similar optimum concentrations of 10(6) to 10(7) cells/mL and may be more general. A mechanism is proposed whereby uPA can associate with binding sites on the cell surface of lower affinity, but higher capacity than uPAR, but these are sufficient to stimulate plasmin generation even at subphysiologic uPA concentrations. This mechanism is likely to operate under conditions commonly used for in vitro studies and may have some significance in vivo.

L17 ANSWER 20 OF 109 MEDLINE on STN

1999257884. PubMed ID: 10326034. Adenovirus-mediated delivery of a uPA/uPAR antagonist suppresses angiogenesis-dependent tumor growth and dissemination in mice. Li H; Lu H; Griscelli F; Opolon P; Sun L Q; Ragot T; Legrand Y; Belin D; Soria J; Soria C; Perricaudet M; Yeh P. (CNRS-Rhone-Poulenc Rorer-IGR UMR 1582, Institut Gustave Roussy, Villejuif, France.) Gene therapy, **(1998 Aug)** 5 (8) 1105-13. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB AdmATF is a recombinant adenovirus encoding a secreted version of the **amino-terminal fragment** (**ATF**) of murine **urokinase** (uPA). This defective adenovirus was used in three murine models to assess the antitumoral effects associated with local or systemic delivery of **ATF**, a broad cell invasion inhibitor that antagonizes uPA binding to its cell surface receptor (uPAR). A single intratumoral injection of AdmATF into pre-established MDA-MB-231 human breast xenografts grown in athymic mice, or into pre-established C57/BL6 syngeneic Lewis lung carcinoma resulted in

a specific arrest of tumor growth. Neovascularization within and at the vicinity of the injection site was also suppressed, suggesting that AdmATF inhibited primary tumor growth by targeting angiogenesis. AdmATF also interfered with tumor cell establishment at distant sites: (1) lung dissemination of Lewis lung carcinoma cells was significantly reduced following intratumoral injection at the primary site; and (2) systemic administration of AdmATF inhibited subsequent liver metastasis in a LS174T human colon carcinoma xenograft model. These data outline the potential of using a recombinant adenovirus directing the secretion of an antagonist of cell-associated uPA for cancer gene therapy.

L17 ANSWER 21 OF 109 MEDLINE on STN

1999163976. PubMed ID: 10066093. **Urokinase** induces receptor mediated brain tumor cell migration and invasion. MacDonald T J; DeClerck Y A; Laug W E. (Department of Pediatrics, Childrens Hospital Los Angeles University of Southern California School of Medicine, 90027, USA.) Journal of neuro-oncology, **(1998 Dec)** 40 (3) 215-26. Journal code: 8309335. ISSN: 0167-594X. Pub. country: Netherlands. Language: English.

AB The plasminogen activation (PA) system plays an important role in tumor invasion by initiating pericellular proteolysis of the extracellular matrix (ECM) and inducing cell migration. Malignant brain tumors overexpress PA members and characteristically invade by migrating on ECM-producing white matter tracts and blood vessel walls. To determine whether **urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) directly modulate the migration of brain tumor cells, we examined six human brain tumor cell lines, 2 astrocytomas (SW1088, SW1783), 2 medullobastomas (Daoy, D341Med), and 2 glioblastomas (U87MG, U118MG), for their surface uPAR expression, endogenous PA activity, and functional proteolytic activity by an ECM-degradation assay. Migration on Transwell membranes and invasion of Matrigel was then tested by pre-incubating the cells with increasing concentrations of either uPA, the proteolytically inactive **amino-terminal fragment** (**ATF**) of uPA, or the uPAR cleaving enzyme, phosphatidylinositol-specific phospholipase C (PI-PLC). All of the cell lines, except D341Med, express surface uPAR protein and uPA activity. High levels of uPAR and uPA activity correlated with cellular degradation of ECM, cell migration, and Matrigel invasion. Cell migration and invasion were enhanced by uPA or **ATF** in a dose dependent manner, while PI-PLC treatment abolished the uPA effect and inhibited migration and invasion. We conclude that ligation of uPAR by uPA directly induces brain tumor cell migration, independent of uPA-mediated proteolysis; and in concert with ECM degradation, markedly enhances invasion. Conversely, removing membrane bound uPAR from the surface of the cells studied inhibited their ability to migrate and invade even in the presence of proteolytically active uPA.

L17 ANSWER 22 OF 109 MEDLINE on STN

1999141290. PubMed ID: 9974409. **Urokinase** activates the Jak/Stat signal transduction pathway in human vascular endothelial cells. Dumler I; Kopmann A; Weis A; Mayboroda O A; Wagner K; Gulba D C; Haller H. (Franz Volhard Clinic and Max-Delbruck Center for Molecular Medicine, Virchow Klinikum-Charite, Humboldt University of Berlin,Germany.. dumler@fvk-berlin.de) . Arteriosclerosis, thrombosis, and vascular biology, **(1999 Feb)** 19 (2) 290-7. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Endothelial cells demonstrate high **urokinase** expression and upregulation of **urokinase** receptors in response to vascular injury. **Urokinase** receptor binding facilitates endothelial cell migration into an arterial wound; however, the signaling cascade induced by the **urokinase** receptor in this cell type is incompletely understood. Because the Janus kinase (Jak)/signal transducer and activator of transcription (Stat) pathway seems to be important for vessel function, we investigated the hypothesis that **urokinase** receptor binding activates Jak/Stat signaling in human vascular endothelial cells. Incubation of endothelial cells with

urokinase-type plasminogen activator (uPA,1 nmol/L) induced a rapid and pronounced increase in tyrosine phosphorylation of several proteins with a molecular weight between 80 to 90 and 130 to 140 kDa. The same pattern of tyrosine phosphorylation was found after treatment with 1 nmol/L **ATF**, the **urokinase amino-terminal fragment**, which is devoid of proteolytic activity but still binds to the **urokinase** receptor. Using coimmunoprecipitation techniques, we demonstrated that the activated **urokinase** receptor is associated with 2 cytoplasmic tyrosine kinases of the Jak family, viz, Jak1 and Tyk2. uPA and **ATF** induced a time-dependent activation of both kinases, as shown by immunoprecipitation and Western blot analysis. Using electrophoretic mobility shift and supershift assays, we then demonstrated that Stat1 is rapidly activated in endothelial cells in response to uPA and **ATF**. Furthermore, Stat1 specifically binds to the regulatory elements interferon-gamma activation site/interferon-stimulated response element. The uPA-induced, time-dependent translocation of Stat1 to cell nuclei was confirmed by confocal microscopy study and immunoblotting of nuclear extracts with an anti-Stat1 antibody. This study provides evidence for a novel signaling pathway for uPA in human vascular endothelial cells. Direct activation of the Jak/Stat system via the uPA-receptor complex may be an important mechanism for endothelial cell migration and/or proliferation during angiogenesis and after vascular injury.

L17 ANSWER 23 OF 109 MEDLINE on STN

1999111061. PubMed ID: 9815812. Vitronectin binding to **urokinase** receptor in human breast cancer. Carriero M V; Del Vecchio S; Franco P; Potena M I; Chiaradonna F; Botti G; Stoppelli M P; Salvatore M. (Istituto Nazionale per lo Studio e la Cura dei Tumori, Via M. Semmola.) Clinical cancer research : an official journal of the American Association for Cancer Research, **(1997 Aug)** 3 (8) 1299-308. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB Functional assembly of the plasminogen-dependent proteolytic system on the cell surface requires multiple interactions involving **urokinase** (uPA), **urokinase** receptor (uPAR), plasminogen activator inhibitors, and other molecules that mediate cell migration and adhesion. We analyzed the in vitro interaction of uPAR-containing particulate cell fractions with the **amino-terminal fragment** (**ATF**) of human **urokinase** and the matrix-like form of vitronectin. Binding and cross-linking of 125I-labeled **ATF** to crude membrane extracts from LB6-19 mouse cells overexpressing human uPARs in the presence of 25 nM urea-denatured vitronectin led to the formation of Mr 137,000, 92, 000, and 82,000 covalent complexes. Immunoprecipitation of the preformed cross-linked 125I-labeled complexes with anti-vitronectin, anti-uPA, or anti-uPAR antibodies revealed that the Mr 82,000 and 92, 000 species do contain **ATF** and vitronectin and identified the Mr 137, 000 species as a ternary complex formed by **ATF**, uPAR, and vitronectin. A similar electrophoretic pattern was displayed by acid-pretreated membranes extracted from MCF-7 breast carcinoma or HT1080 fibrosarcoma cell lines, as well as a ductal breast carcinoma specimen; the latter exhibited complex formation at concentrations of vitronectin lower than 10 nM. Finally, uPAR-vitronectin interaction was further documented by the decreased reactivity of an anti-uPAR polyclonal antibody to acid-pretreated sections of 10 breast carcinomas that had been preincubated with vitronectin. Our findings highlight the ability of uPAR to interact simultaneously with vitronectin and uPA in breast cancer, supporting a dynamic coupling of the molecular mechanisms underlying plasminogen-dependent matrix degradation and cell adhesion.

L17 ANSWER 24 OF 109 MEDLINE on STN

1999101360. PubMed ID: 9886269. Suppression of keratinocyte proliferation by plasminogen activator inhibitor-2. Hibino T; Matsuda Y; Takahashi T; Goetinck P F. (Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School,

Charlestown, USA.) Journal of investigative dermatology, **(1999 Jan)** 112 (1) 85-90. Journal code: 0426720. ISSN: 0022-202X. Pub. country: United States. Language: English.

AB We have previously shown that **urokinase** plasminogen activator (uPA) stimulates the growth of human keratinocytes in culture. For this effect, uPA activity is essential to generate the active **amino terminal fragment**, by an autolytic process. Our findings indicated further that inhibition of uPA may result in the suppression of growth of keratinoytes. Here, we provide evidence that plasminogen activator inhibitor (PAI)-2 has an anti-proliferative effect on keratinocytes. The uPA activity in cultured keratinocytes increased in parallel with cell proliferation, reaching a maximum level at confluency and decreasing gradually thereafter. The analysis of synchronized cells showed that the peak uPA activity in the medium occured just prior to S-phase, suggesting that the production and secretion of uPA is related to cell proliferation. In contrast, PAI-2 levels showed a steady increase, even after confluency. When PAI-2, purified from human cornified cells, was added to synchronized keratinocytes, S-phase was no longer evident and the peak uPA activity was eliminated. In experiments with a bacterially expressed PAI-2 fusion protein, [3H]thymidine incorporation by keratinocytes was significantly suppressed, confirming an anti-proliferative effect of PAI-2. These results strongly suggest that PAI-2 is involved in the regulation of keratinocyte proliferation and differentiation.

L17 ANSWER 25 OF 109 MEDLINE on STN

1999087634. PubMed ID: 9872599. Plasminogen activator system modulates invasive capacity and proliferation in prostatic tumor cells. Festuccia C; Dolo V; Guerra F; Violini S; Muzi P; Pavan A; Bologna M. (Department of Experimental Medicine, University of L'Aquila, Italy.) Clinical & experimental metastasis, **(1998 Aug)** 16 (6) 513-28. Journal code: 8409970. ISSN: 0262-0898. Pub. country: Netherlands. Language: English.

AB The malignant phenotype of prostatic tumor cells correlates with the expression of both uPA and its cell-membrane receptor (uPAR); however, there is little information concerning the role of cell-bound uPA in matrix degradation and invasion. Our results suggest that cell-associated uPA plays a key role in regulating the amount of plasmin present at the surface of prostatic carcinoma (PRCA) cells and show that differential production of uPA corresponds with the capacity to bind and activate plasminogen. In addition, we provide direct evidence that both uPA secretion and the presence of uPA-uPAR complexes characterize the invasive phenotype of PRCA cells and suggest the existence of several pathways by which tumor cells acquire plasmin activity. LNCaP cells (which do not produce uPA but express uPAR) may activate plasmin through exogenous uPA. In vivo, the source of uPA may be infiltrating macrophages and/or fibroblasts as observed in several other systems. PAI-1 accumulation in the conditioned medium (CM) limits plasmin action to the pericellular microenvironment. Our results indicate that MMP-9 and MMP-2 are also activated by plasmin generated by cell-bound but not by soluble, extracellular uPA. Plasmin activation and triggering of the proteolytic cascade involved in Matrigel invasion is blocked by antibodies against uPA (especially by anti- A-chain of uPA which interacts with uPAR) and by PA inhibitors such as p-aminobenzamidine which may regulate levels of cell-bound uPA. uPA may also regulate growth in PRCA cells. Indeed, antibodies against uPA A-chain (and also p-aminobenzamidine treatment) interfere with the **ATF** domain and inhibit cell growth in uPA-producing PC3 and DU145 prostate cancer cell lines, whereas exogenous uPA (**HMW-uPA** with **ATF**) induces growth of LNCaP prostate tumor cell line. These data support the hypothesis that in prostatic cancer patients at risk of progression, uPA/plasmin blockade may be of therapeutic value by blocking both growth of the primary tumor and dissemination of metastatic cells.

L17 ANSWER 26 OF 109 MEDLINE on STN

1999065689. PubMed ID: 9848876. **Urokinase** receptor-dependent upregulation of smooth muscle cell adhesion to vitronectin by **urokinase**. Chang A W; Kuo A; Barnathan E S; Okada S S. (University of Pennsylvania School of Medicine, Philadelphia, USA.) Arteriosclerosis, thrombosis, and vascular biology, **(1998 Dec)** 18 (12) 1855-60. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB The plasminogen activator system has been implicated in the modulation of the response to vascular injury. Although **urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) may enhance matrix degradation as well as migration and invasion by smooth muscle cells (SMCs), their roles in cell adhesion are uncertain. Therefore, we examined the ability of uPA and uPAR to modulate adhesion of cultured human vascular SMCs to various matrices. We demonstrated a dose-dependent stimulation of adhesion by single-chain uPA (scuPA) to vitronectin (maximum 1.55-fold [+/-0. 04-fold] increase, 10 nmol/L, P<0.002) but not to laminin, collagen I, or collagen IV. Baseline adhesion to vitronectin was completely inhibited by both EDTA and RGD peptide but was restored to >40% of control in the presence of scuPA (P=0.001 and 0.046, respectively). Adhesion to vitronectin was also significantly enhanced by the **amino-terminal fragment** of uPA (P=0.007) and two-chain, high-molecular-weight uPA (P<0.01) but not by the low-molecular-weight fragment of uPA, which lacks the receptor-binding domain. Aprotinin, a plasmin inhibitor, had no effect on baseline or scuPA-stimulated adhesion, suggesting a plasmin-independent process. Preincubation of scuPA with soluble uPAR inhibited scuPA stimulation of adhesion by 88+/-14% (P=0.01), as did pretreatment of SMCs with phosphatidylinositol-specific phospholipase C, which removes glycophosphatidylinositol-anchored proteins, including uPAR. Antibodies to both alphavbeta3 and alphavbeta5 integrin inhibited baseline adhesion but not scuPA stimulation. Finally, coating plates with scuPA alone enabled cell adhesion, which could be inhibited by both soluble uPAR and anti-uPAR antibodies. These data suggest that uPA stimulates adhesion of SMCs specifically to vitronectin and that it is mediated by an interaction with uPAR. Upregulation of both proteins after vascular injury may facilitate migration through stimulation of both matrix degradation and cell adhesion.

L17 ANSWER 27 OF 109 MEDLINE on STN

1999057882. PubMed ID: 9837898. Mitogenic effects of **urokinase** on melanoma cells are independent of high affinity binding to the **urokinase** receptor. Koopman J L; Slomp J; de Bart A C; Quax P H; Verheijen J H. (Gaubius Laboratory, TNO Prevention and Health, 2301 CE Leiden, The Netherlands.) Journal of biological chemistry, **(1998 Dec 11)** 273 (50) 33267-72. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The structural and functional properties of the **urokinase**-type plasminogen activator (u-PA) that are involved in the mitogenic effect of this proteolytic enzyme on human melanoma cells M14 and IF6 and the role of the u-PA receptor (u-PAR) in transducing this signal were analyzed. Native u-PA purified from urine induced a mitogenic response in quiescent IF6 and M14 cells that ranged from 25 to 40% of the mitogenic response obtained by fetal calf serum. The half-maximum response in M14 and IF6 cells was reached at u-PA concentrations of approximately 35 and 60 nM, respectively. Blocking the proteolytic activity of u-PA resulted in a 30% decrease of the mitogenic effect, whereas inhibition of plasmin activity did not alter the mitogenic effect. No mitogenic response was elicited by low molecular weight u-PA, lacking the growth factor domain and the kringle domain. The **ATF** domain of u-PA induced a mitogenic response that was similar to complete u-PA. Defucosylated **ATF** and recombinant u-PA purified from Escherichia coli lacking all post-translational modifications did not induce a mitogenic response. Blocking the interaction of u-PA with u-PAR, using a specific monoclonal antibody, did not alter the mitogenic effect induced by u-PA. The binding of radiolabeled u-PA to M14 and IF6 cells was characterized by high affinity

binding mediated by u-PAR and low affinity binding to an unknown binding site. These results demonstrate that proteolytically inactive u-PA is able to induce a mitogenic response in quiescent melanoma cells in vitro by a mechanism that involves the **ATF** domain but is independent of high affinity binding to u-PAR. Furthermore, it suggests that u-PA is able to bind with low affinity to a hitherto unidentified membrane associated protein that could be involved in u-PA-induced signal transduction.

L17 ANSWER 28 OF 109 MEDLINE on STN

1999037729. PubMed ID: 9821967. **Urokinase** induces proliferation of human ovarian cancer cells: characterization of structural elements required for growth factor function. Fischer K; Lutz V; Wilhelm O; Schmitt M; Graeff H; Heiss P; Nishiguchi T; Harbeck N; Kessler H; Luther T; Magdolen V; Reuning U. (Frauenklinik der Technischen Universitat Munchen, Klinikum rechts der Isar, Munich, Germany.) FEBS letters, **(1998 Oct 30)** 438 (1-2) 101-5. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Ovarian cancer metastasis is associated with an increase in the **urokinase**-type plasminogen activator (uPA) and its receptor uPAR. We present evidence that binding of uPA to uPAR provokes a mitogenic response in the human ovarian cancer cell line OV-MZ-6 in which endogenous uPA production had been significantly reduced by stable uPA 'antisense' transfection. High molecular weight (**HMW**) **uPA**, independent of its enzymatic activity, produced an up to 95% increase in cell number concomitant with 2-fold elevated [3H]thymidine incorporation as did the catalytically inactive but uPAR binding **amino-terminal fragment** of uPA, **ATF**. uPA-induced cell proliferation was significantly decreased by blocking uPA/uPAR interaction by the monoclonal antibody IIIF10 and by soluble uPAR. The efficiency of the uPAR binding synthetic peptide cyclo19,31 uPA19-31 to enhance OV-MZ-6 cell growth proved this molecular domain to be the minimal structural determinant for uPA mitogenic activity. Dependence of uPA-provoked cell proliferation on uPAR was further demonstrated in Raji cells which do not express uPAR and were thus not induced by uPA. However, upon transfection with full-length uPAR, Raji cells acquired a significant growth response to **HMW uPA** and **ATF**.

L17 ANSWER 29 OF 109 MEDLINE on STN

1998430982. PubMed ID: 9760182. Analysis of the ternary complex formation of human **urokinase** with the separated two domains of its receptor. Oda M; Shiraishi A; Hasegawa M. (Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd, Japan.. masayuki.oda@kyowa.co.jp) . European journal of biochemistry / FEBS, **(1998 Sep 1)** 256 (2) 411-8. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Human **urokinase**-type-plasminogen-activator receptor (uPAR) is a glycolipid-anchored membrane glycoprotein comprising three structurally similar domains. We have succeeded in direct observation of the ternary complex formation of single-chain **urokinase** (scuPA) or its N-terminal fragment (**ATF**) with the separated domain-1 (N-terminal domain) and domain-(2+3) (internal and C-terminal domain) of human uPAR, by means of gel-filtration HPLC analysis. This complex was found to consist of the three components in an equimolar ratio (thus referred to as the three-part complex). To determine the nature of the interaction between these components, cross-linking experiments involving various kinds of cross-linkers and competitive binding assay on ELISA were performed. These experiments have shown that each uPAR domain can bind directly to scuPA at low affinity, and that both these domains contribute to the high-affinity binding between scuPA and uPAR in a synergistic manner. It can be considered that the synergistic effect of domain-1 and domain-(2+3) on scuPA binding would result from a conformational change, and that this steric event might trigger the signal transduction reported for scuPA/uPAR binding.

L17 ANSWER 30 OF 109 MEDLINE on STN
1998316656. PubMed ID: 9654084. A bifunctional hybrid molecule of the **amino-terminal fragment** of **urokinase** and domain II of bikunin efficiently inhibits tumor cell invasion and metastasis. Kobayashi H; Sugino D; She M Y; Ohi H; Hirashima Y; Shinohara H; Fujie M; Shibata K; Terao T. (Department of Obstetrics and Gynecology, Hamamatsu University, Shizuoka, Japan.) European journal of biochemistry / FEBS, **(1998 May 1)** 253 (3) 817-26. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Urinary trypsin inhibitor (UTI) inhibits efficiently tumor cell invasion and the formation of metastasis. The anti-metastatic effect is dependent on the COOH-terminal domain II of UTI [UTI-(78-136)-peptide]. To develop a molecule that binds with high affinity to the **urokinase** (uPA) receptor (uPAR) on tumor cell surfaces, a bifunctional hybrid molecule [uPA-(1-134)-UTI-(78-136)] consisting of the uPAR-binding NH2-terminal fragment [UTI-(78-136)-peptide] of uPA at the NH2-terminus of UTI-(78-136)-peptide was produced in Escherichia coli by genetic engineering. The purified hybrid protein inhibited trypsin and plasmin 2-3-fold less effectively than UTI-(78-136)-peptide and was found to bind to human tumor cells via uPAR, which was confirmed by cell binding and competition experiments. Using a modified Boyden chamber and an artificial basement membrane, Matrigel, it was found that the hybrid protein is very effective at inhibiting invasion by uPAR-expressing human tumor cells. Sensitivities of tumor cells towards the anti-invasive effect of uPA-(1-134)-UTI-(78-136) correlated with the density of uPAR on human tumor cells. Furthermore, in the spontaneous metastasis model, the hybrid protein inhibited the formation of lung and/or lymphatic metastasis by human ovarian carcinoma and choriocarcinoma cells. The hybrid protein was much more effective than uPA-(1-134)-peptide, UTI-(78-136)-peptide, or UTI. We conclude that this approach extends the possibility of applying recombinant protein for therapeutic use in inhibition of human tumor cell metastasis.

L17 ANSWER 31 OF 109 MEDLINE on STN
1998231068. PubMed ID: 9569610. Production of a hybrid protein consisting of the N-terminal fragment of **urokinase** and the C-terminal domain of urinary trypsin inhibitor in Escherichia coli. Sugino D; Okushima M; Kobayashi H; Terao T. (Nissin Central Research Institute, Shiga, Japan.) Biotechnology and applied biochemistry, **(1998 Apr)** 27 (Pt 2) 145-52. Journal code: 8609465. ISSN: 0885-4513. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We have constructed a hybrid protein (ATFHI) consisting of an N-terminal fragment from **urokinase** (**ATF**) and HI-8, which is the C-terminal domain of urinary trypsin inhibitor. The fusion genes for the hybrid proteins were engineered by PCR and cloned into expression plasmids. Under the control of the tac promoter, fusion genes were efficiently expressed in Escherichia coli. The hybrid proteins, produced as inclusion bodies in E. coli, were refolded by a dialysis method and purified by ion-exchange chromatography. ATFHI exhibited bifunctional activity related to antimetastatic effects: the **urokinase** receptor-binding activity of **ATF** and the inhibitory activity of HI-8 on plasmin.

L17 ANSWER 32 OF 109 MEDLINE on STN
1998192651. PubMed ID: 9525964. Binding of **urokinase**-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal-regulated kinase 1 and 2 which is required for increased cellular motility. Nguyen D H; Hussaini I M; Gonias S L. (Department of Biochemistry, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA.) Journal of biological chemistry, **(1998 Apr 3)** 273 (14) 8502-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Binding of **urokinase**-type plasminogen activator (uPA) to its receptor, uPAR, regulates cellular adhesion, migration, and tumor cell invasion.

Some of these activities may reflect the ability of uPAR to initiate signal transduction even though this receptor is linked to the plasma membrane only by a glycosylphosphatidylinositol anchor. In this study, we demonstrated that single-chain uPA activates extracellular signal-regulated kinase 1 (ERK1) and ERK2 in MCF-7 breast cancer cells. Phosphorylation of ERK1 and ERK2 was increased 1 min after adding uPA and returned to baseline levels by 5 min. The **amino-terminal fragment** (**ATF**) of uPA, which binds to uPAR but lacks proteinase activity, also activated ERK1 and ERK2. Responses to uPA and **ATF** were eliminated when the cells were pretreated with PD098059, an inhibitor of mitogen-activated protein kinase kinase. uPA and **ATF** promoted the migration of MCF-7 cells across serum-coated Transwell membranes in vitro. Migration was increased 2.1 +/- 0.4-fold when uPA was added to the top chamber, 4. 8 +/- 0.8-fold when uPA was added to the bottom chamber, and 7.7 +/- 1.0-fold when uPA was added to both chambers. MCF-7 cells that were pulse-exposed to uPA for 30 min, and then washed to remove unbound ligand, demonstrated increased motility even though migration was allowed to occur for 24 h. PD098059 completely neutralized the effects of uPA on MCF-7 cellular motility, irrespective of whether the uPA was present for the entire motility assay or administered by pulse-exposure. These results demonstrate a novel, receptor-dependent signaling activity which is required for uPA-stimulated breast cancer cell migration.

L17 ANSWER 33 OF 109 MEDLINE on STN

1998073730. PubMed ID: 9409265. Induction of vascular SMC proliferation by **urokinase** indicates a novel mechanism of action in vasoproliferative disorders. Kanse S M; Benzakour O; Kanthou C; Kost C; Lijnen H R; Preissner K T. (Max-Planck-Institute, Kerckhoff-Klinik, Bad Nauheim, Germany.. sandip.kanse@kerckhoff.med.uni-giessen.de) . Arteriosclerosis, thrombosis, and vascular biology, **(1997 Nov)** 17 (11) 2848-54. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB The **urokinase**-type plasminogen activator (UPA) and its receptor are expressed in the vasculature and are involved in cell migration and remodeling of the extracellular matrix in the neointima. Vessels with atherosclerosis or neointimal hyperplasia, when compared with normal vessels, contain high UPA activity as well as increased levels of UPA receptor. In this study, we have identified the stimulation of vascular smooth muscle cell proliferation as a novel activity for UPA in the vessel wall. High-molecular-weight-UPA (12-200 nmol/L range) stimulated DNA synthesis and cell proliferation, which was half that induced by fetal calf serum or by platelet-derived growth factor-BB. UPA did not induce growth of endothelial cells, and tissue-type plasminogen activator showed no activity on either cell type. Induction of proliferation required the complete UPA molecule but was independent of the proteolytic activity of UPA, whereas neither the **amino-terminal fragment** nor the catalytic domain by itself was mitogenic. UPA also stimulated c-fos/c-myc mRNA expression and mitogen-activated protein kinase activity in smooth muscle cells. Blocking monoclonal antibodies against the UPA receptor and the enzymatic removal of receptors were ineffective in inhibiting the mitogenic effect of UPA, suggesting a UPA receptor-independent mechanism. Thus, we provide evidence for a novel function of UPA on vascular smooth muscle cell proliferation that, together with its previously documented involvement in regulating pericellular proteolysis-related events and cell migration, provides additional evidence for a role in the pathogenesis of atherosclerosis/restenosis.

L17 ANSWER 34 OF 109 MEDLINE on STN

1998072443. PubMed ID: 9409785. Cooperation of two PEA3/AP1 sites in uPA gene induction by TPA and FGF-2. D'Orazio D; Besser D; Marksitzer R; Kunz C; Hume D A; Kiefer B; Nagamine Y. (Friedrich Miescher Institute, Basel, Switzerland.) Gene, **(1997 Nov 12)** 201 (1-2) 179-87. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB We have previously shown in NIH 3T3 fibroblasts that treatment with 12-O-tetradecanoylphorbol 13-acetate (TPA) or fibroblast growth factor-2 (FGF-2) activates the Ras/Erk signaling pathway in NIH 3T3 fibroblasts, leading to the induction of the **urokinase**-type plasminogen activator (uPA) gene. In this study, we characterize cis-acting elements involved in this induction. DNase I hypersensitive (HS) site analysis of the uPA promoter showed that two regions were enhanced after TPA and FGF-2 treatment. One was located 2.4kb upstream of the transcription start site (-2.4kb), where a known PEA3/AP1 (AGGAAATGAGGTCAT) element is located. The other was located in a previously undefined far upstream region. Sequencing of this region revealed a similar AP1/PEA3 (GTGATTCACTTCCT) element at -6.9 kb corresponding to the HS site. Deletion analysis of the uPA promoter in transient transfection assays showed that both PEA3/AP1 elements are required for full inducibility, suggesting a synergism between the two elements. When the two sites were inserted together upstream of a minimal promoter derived from the thymidine kinase gene, expression of the reporter gene was more strongly induced by TPA and FGF-2 than with either of the two elements alone. Alone, the -6.9 element was more potent than the -2.4 element. The involvement of AP1 as well as Ets transcription factors was confirmed by examining different promoter constructs containing deletions in either the AP-1 or the PEA3 element, and by using an expression plasmid for dominant negative Ets-2. Electromobility shift analyses using specific antibodies showed that c-Jun and, JunD bind to both elements with or without induction. In addition, **ATF**-2 binds to the -2.4-kb element even without induction and c-Fos to the -6.9-kb element only after induction. Accordingly, overexpression of c-Fos caused induction from the -6.9-kb element, but reduced induction from the -2.4-kb element. The involvement of the Ets-2 transcription factor was shown by using expression plasmids for wild-type and dominant negative Ets-2.

L17 ANSWER 35 OF 109 MEDLINE on STN

1998033175. PubMed ID: 9367352. The **amino-terminal fragment** of human **urokinase** directs a recombinant chimeric toxin to target cells: internalization is toxin mediated. Fabbrini M S; Carpani D; Bello-Rivero I; Soria M R. (Dibit-Department of Biological and Technological Research, San Raffaele Scientific Institute, Milano, Italy.. fabbris@dibit.hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, **(1997 Nov)** 11 (13) 1169-76. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB In contrast to two-chain **urokinase** (uPA), a chemical conjugate between uPA and native saporin (a cytotoxic plant seed ribosome-inactivating protein) did not require plasminogen activator inhibitors to be internalized. To dissect this pathway, we constructed a chimera consisting of the **amino-terminal fragment** (**ATF**) of human **urokinase** fused to a saporin isoform (SAP-3). The chimeric **ATF**-SAP toxin was expressed in Escherichia coli, purified, and characterized for its ribosome-inactivating activity. Besides being a potent inhibitor of protein synthesis in cell-free assays, **ATF**-SAP was specifically cytotoxic toward cells expressing human uPAR. Competition experiments indicated that both the human uPAR and the LDL-related receptor protein are involved in mediating the cell killing ability of **ATF**-SAP. We conclude that neither plasminogen activator inhibitors nor the catalytic moiety of **urokinase** are necessary to initiate these internalization pathways. Thus, saporin may play a role similar to plasminogen activator inhibitors in its ability to trigger internalization of uPAR-bound ligands through endocytic receptors.

L17 ANSWER 36 OF 109 MEDLINE on STN

1998026748. PubMed ID: 9362425. Mechanisms of the development of osteoblastic metastases. Goltzman D. (Department of Medicine, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada.)

Cancer, **(1997 Oct 15)** 80 (8 Suppl) 1581-7. Ref: 29. Journal code: 0374236. ISSN: 0008-543X. Pub. country: United States. Language: English.

AB Although several neoplasms may produce osteoblastic metastases, carcinoma of the prostate is by far the most common. Biochemical and histologic studies indicate that osteolysis also is a manifestation of prostate carcinoma. Furthermore, factors such as parathyroid hormone-related peptide, which mediate osteolysis in other cancers, also appear to be operative in the bone breakdown induced by prostate carcinoma. However, the most unique skeletal effect of this tumor is its consistent capacity to stimulate osteoblasts to deposit new bone. Several bone growth factors have been detected in prostatic tissue and may contribute to this process. These include transforming growth factor-beta, fibroblast growth factor, and bone morphogenetic proteins. The author isolated an **amino-terminal fragment** (**ATF**) of the protease **urokinase** (uPA) from the conditioned medium of the prostate carcinoma cell line PC-3 and demonstrated that this fragment has mitogenic activity for osteoblastic cells. The activity appears to reside in an epidermal growth factor-like growth factor domain (GFD) within the **ATF**. Subsequently, the author cloned the rat uPA receptor (uPAR). uPAR is known to bind the **ATF** and can permit the uPA molecule to exhibit focal proteolysis. It was shown that the **ATF** also can induce c-myc, c-jun, and c-fos in osteoblastic cells. This effect of **ATF** can be mimicked by the GFD and suggests that this signalling pathway in osteoblasts is via the uPAR. Consequently, the uPA molecule may contribute to growth factor effects in osteoblasts via the NH2-terminal fragment and to tumor invasiveness via its COOH-terminal proteolytic domain. This scenario is supported by results from studies with uPA-overexpressing prostate carcinoma cells in rats. Additional studies will be required to further define the mechanisms of interaction of prostate carcinoma and other cancers with bone but each site of molecular interaction may provide a therapeutic window for curtailing the effects of these tumors on the skeleton.

L17 ANSWER 37 OF 109 MEDLINE on STN

97415430. PubMed ID: 9271229. Defective cell migration in an ovarian cancer cell line is associated with impaired **urokinase**-induced tyrosine phosphorylation. Mirshahi S S; Lounes K C; Lu H; Pujade-Lauraine E; Mishal Z; Benard J; Bernadou A; Soria C; Soria J. (Laboratoire Sainte Marie, Hotel Dieu, Parvis de Notre Dame, Paris, France.) FEBS letters, **(1997 Jul 14)** 411 (2-3) 322-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The **urokinase** receptor (u-PAR), a protein anchored to cell membrane by a glycosyl phosphatidylinositol, plays a central role in cancer cell invasion and metastasis by binding **urokinase** plasminogen activator (u-PA), thereby facilitating plasminogen activation. Plasmin can promote cell migration either directly or by activating metalloproteinases that degrade some of the components of the extra cellular matrix. However, the IGR-OV1-Adria cell line contains the u-PAR but does not migrate even in the presence of exogenous u-PA, although the parental IGR-OV1 cell line migrates normally in the presence of u-PA. We therefore investigated the role of cell signalling for u-PA induced cell locomotion. We show that cell migration induced by u-PA-u-PAR complex is always associated with tyrosine kinase activation for the following reasons: (1) the blockade of the u-PAR by a chimeric molecule (albumin-**ATF**) inhibits not only the u-PA-induced cell migration, but also the signalling in IGR-OV1 line; (2) the binding of u-PA to u-PAR on non-migrating IGR-OV1-Adria cells was not associated with tyrosine kinase activation; (3) the inhibition of tyrosine kinase also blocked cell migration of IGR-OV1. Therefore tyrosine kinase activation seems to be essential for the u-PA-induced cell locomotion possibly by the formation of a complex u-PAR-u-PA with a protein whose transmembrane domain can ensure cell signalling. Thus, IGR-OV1 and IGR-OV1-Adria cell lines represent a good model for the analysis of the mechanism of u-PA-u-PAR-induced cell locomotion.

L17 ANSWER 38 OF 109 MEDLINE on STN
97402957. PubMed ID: 9258335. Induction in human osteoblastic cells (SaOS2) of the early response genes fos, jun, and myc by the **amino terminal fragment** (**ATF**) of **urokinase**. Rabbani S A; Gladu J; Mazar A P; Henkin J; Goltzman D. (Department of Medicine, McGill University, Montreal, Quebec, Canada.) Journal of cellular physiology, **(1997 Aug)** 172 (2) 137-45. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Previous studies have demonstrated that overexpression of urinary plasminogen activator (uPA) in rat prostate cancer cells results in increased skeletal metastases, which are primarily of the osteoblastic variety. The osseous activation induced by the metastases appears to be mediated through the **amino terminal fragment** (**ATF**) of uPA, which lacks the catalytic domain and can act as a growth factor for osteoblasts. To explore further the mechanism of action of uPA in bone cells, we evaluated the effects of **ATF** on modulating the expression of various proto-oncogenes. Human-osteoblast-derived osteosarcoma cells, SaOS2, were treated with graded doses of **ATF** for 10-120 min, and effects on early response proto-oncogenes were monitored. **ATF** increased c-myc, c-jun, and c-fos gene expression in a time-dependent manner for up to 60 min, after which mRNA levels fell. The maximum induction was seen in c-fos gene expression, which was found to be dose dependent. This effect of **ATF** was localized to its growth-factorlike domain. Examination of the half life of these transcripts in the presence of the transcriptional inhibitor actinomycin D demonstrated that **ATF** does not alter the stability of c-fos mRNA in these bone cells. Nuclear run-off assays indicated that **ATF** effects were due to stimulation of c-fos gene transcription. An increase in c-fos protein levels was correlated with the augmentation of its mRNA in **ATF**-treated SaOS2 cells. Pretreatment of SaOS2 cells with the protein tyrosine kinase inhibitor herbimycin and recombinant soluble uPA receptor (uPAR) caused a significant reduction in the ability of **ATF** to induce c-fos expression. These results demonstrate a novel role for uPA in activating early response proto-oncogenes, in particular c-fos, which plays an important role in bone cell growth and differentiation and may be a key factor in the signal transduction pathway of **ATF**.

L17 ANSWER 39 OF 109 MEDLINE on STN
97318767. PubMed ID: 9175704. Evidence of a non-conventional role for the **urokinase** tripartite complex (uPAR/uPA/PAI-1) in myogenic cell fusion. Bonavaud S; Charriere-Bertrand C; Rey C; Leibovitch M P; Pedersen N; Frisdal E; Planus E; Blasi F; Gherardi R; Barlovatz-Meimon G. (Groupe d'Etudes et de Recherches sur le Muscle et le Nerf (GERMEN: ER 269+ 315), Universite Paris XII, Creteil, France.) Journal of cell science, **(1997 May)** 110 (Pt 9) 1083-9. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Urokinase** can form a tripartite complex binding **urokinase** receptor (uPAR) and plasminogen activator inhibitor type-1 (PAI-1), a component of the extracellular matrix (ECM). The components of the tripartite complex are modulated throughout the in vitro myogenic differentiation process. A series of experiments aimed at elucidating the role of the **urokinase** tripartite complex in the fusion of human myogenic cells were performed in vitro. Myogenic cell fusion was associated with increased cell-associated **urokinase**-type plasminogen activator (uPA) activity, cell-associated uPAR, and uPAR occupancy. Incubation of cultures with either uPA anticatalytic antibodies, or the **amino-terminal fragment** of uPA (**ATF**), which inhibits competitively uPA binding to its receptor, or anti-PAI-1 antibodies, which inhibit uPA binding to PAI-1, resulted in a 30 to 47% decrease in fusion. Incubation of cultures with the plasmin inhibitor aprotinin did not affect fusion. Decreased fusion rates induced by interfering with uPAR/uPA/PAI-1 interactions were not associated with significant changes in mRNA levels of both the myogenic regulatory factor myogenin and its inhibitor of DNA binding, Id. Incubation of cultures

with purified uPA resulted in a decrease in fusion, likely due to a competitive inhibition of PAI-1 binding of endogenous uPA. We conclude that muscle cell fusion largely depends on interactions between the members of the **urokinase** complex (uPAR/uPA/PAI-1), but does not require proteolytic activation of plasmin. Since the intrinsic muscle cell differentiation program appears poorly affected by the state of integrity of the **urokinase** complex, and since cell migration is a prerequisite for muscle cell fusion in vitro, it is likely that the **urokinase** system is instrumental in fusion through its connection with the cell migration process. Our results suggest that the **urokinase** tripartite complex may be involved in cell migration in a non conventional way, playing the role of an adhesion system bridging cell membrane to ECM.

L17 ANSWER 40 OF 109 MEDLINE on STN

97286953. PubMed ID: 9142045. Beta 2 (CD11/CD18) integrins can serve as signaling partners for other leukocyte receptors. Todd R F 3rd; Petty H R. (Department of Internal Medicine, University of Michigan Medical School, Ann Arbor 48109-0374, USA.) Journal of laboratory and clinical medicine, **(1997 May)** 129 (5) 492-8. Ref: 64. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB Fig. 1 depicts our current thinking about the ways in which Mo1 and p150,95 form cis interactions with other leukocyte receptors. With respect to the associations of Mo1 with Fc gamma RIIIB and uPAR, the inhibitory effect of saccharides such as NADG suggests a lectin-carbohydrate interaction that may involve the recognition of Mo1's beta-glucan site for N-linked carbohydrates4 that are expressed by both Fc gamma RIIIB and uPAR. This hypothesis is supported by the results of Stockl et al., who showed that the binding of C-terminal-specific mAb VIM12 to Mo1, which enhances the phospholipase C-mediated release of Fc gamma RIIIB, was inhibited by NADG. However, unlike the sample lectin-carbohydrate interaction that appears to govern the association between Mo1 and Fc gamma RIIIB, effective Mo1-dependent uPAR signaling also depends on the binding of intact uPA to uPAR (the receptor-binding **ATF** of uPA proving insufficient to prime neutrophils for an enhanced burst response to FMLP). We speculate that **ATF** (residues 6-135) binds to uPAR while the carboxyl terminal fragment (residues 136-411), which includes a glycosylation site at residue 144, binds to the lectinlike site of Mo1, thus fostering the linkage between the two receptors. In support of this model is the fact that exposure of neutrophils to **ATF** reduced the degree of molecular proximity between Mo1 and uPAR (the latter probably occupied by endogenous intact uPA) and increased the molecular association between Mo1 and Fc gamma RIIIB (both as detected by quantitative RET). This hypothesis is analogous to the concept proposed by Nykjaer et al in which plasminogen activator inhibitor-1 initially binds to uPA to form a complex that secondarily binds to the alpha 2 macroglobulin receptor, leading to internalization of the complex. Whereas the contribution of intact uPA to the interaction between Mo1 and uPAR remains speculative (based on the indirect data available), no such ambiguity exists for the role of the LPS/LBP ligand in regulating the association between Mo1 and CD14. In this circumstance, no physical linkage exists between the two receptors without the ligand complex. This observation is consistent with the previously described affinity of the beta 2 integrins for LPS, leading to the notion that the LPS portion of the LPS/LPB complex binds to Mo1, serving to link it with LPS/LBP bound to CD14. The observed reversibility of the interactions between the integrin glycoproteins and uPAR or CD14 illustrates the fact that these associations can be highly dynamic and tied to cellular processes that include directed motility (Mo1-uPAR), adherence to substrates (Mo1-CD14), and energy metabolism (p150,95-uPAR). We speculate that the GPI-anchored receptor proteins serve as rapidly diffusible, expendable "scouts" for the beta 2 integrins, which serve to expand their ligand binding repertoire in a cis-acting fashion.

L17 ANSWER 41 OF 109 MEDLINE on STN

97250991. PubMed ID: 9096674. Melanoma cell migration on vitronectin: regulation by components of the plasminogen activation system. Stahl A; Mueller B M. (Department of Immunology, Scripps Research Institute, La Jolla, CA 92037, USA.) International journal of cancer. Journal international du cancer, **(1997 Mar 28)** 71 (1) 116-22. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Tumor cell migration and invasion require complex interactions between tumor cells and the surrounding extracellular matrix. These interactions are modified by cell adhesion receptors, as well as by proteolytic enzymes and their receptors. Here, we study the influence of the protease urokinasetype plasminogen activator (uPA) and its receptor (uPAR) on melanoma cell adhesion to, and migration on, the extracellular matrix protein vitronectin (VN). Cell adhesion to VN, but not to type I collagen, is significantly enhanced in the presence of either uPA or its **amino-terminal fragment** (**ATF**). Soluble uPAR can inhibit this effect, indicating that uPA/uPAR on melanoma cells can function as a VN receptor. In the absence of bivalent cations, uPA/uPAR can promote cell attachment on VN, but not cell spreading, suggesting that the glycosylphosphatidylinositol (GPI)-anchored uPAR alone is unable to organize the cytoskeleton. Chemotactic melanoma cell migration on a uniform VN matrix is inhibited by uPA and **ATF**, implying that cell motility decreases when uPA/uPAR acts as a VN receptor. In contrast, plasminogen activator inhibitor I (PAI-I) can stimulate melanoma cell migration on VN, presumably by inhibiting uPA/uPAR-mediated cell adhesion to VN and thereby releasing the inhibition of cell migration induced by uPA. Together, our data implicate components of the plasminogen activation system in the direct regulation of cell adhesion and migration, thereby modulating the behavior of malignant tumor cells.

L17 ANSWER 42 OF 109 MEDLINE on STN

97228369. PubMed ID: 9117184. Bone matrix degradation by the plasminogen activation system. Possible mechanism of bone destruction in arthritis. Ronday H K; Smits H H; Quax P H; van der Pluijm G; Lowik C W; Breedveld F C; Verheijen J H. (Department of Vascular and Connective Tissue Research, Gaubius Laboratory, TNO-PG, Leiden, The Netherlands.) British journal of rheumatology, **(1997 Jan)** 36 (1) 9-15. Journal code: 8302415. ISSN: 0263-7103. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The observed increase in **urokinase**-type plasminogen activator (u-PA) and its receptor (u-PAR) in synovial tissue of patients with rheumatoid arthritis (RA) suggests pathophysiological involvement of the plasminogen activation (PA) system in inflammatory joint disease. In the present study, we investigated the capacity of the PA system to degrade non-mineralized and mineralized bone-like matrix in vitro as a model for bone destruction. Transfected mouse LB6 cell lines, that expressed either human u-PA or u-PAR, were cultured separately and simultaneously on radiolabelled bone matrix in the presence of plasminogen. Osteoblast-like murine calvarial MC3T3-E1 cells were used to produce a well-characterized, highly organized bone-like matrix, that could be mineralized in the presence of beta-glycerol phosphate. Bone matrix degradation was followed by the release of radioactivity in the culture medium. u-PA-producing cells, in contrast to u-PAR-producing cells, degraded both non-mineralized and mineralized bone matrix. This effect could be inhibited by anti-u-PA antibodies, as well as by tranexamic acid and by aprotinin, indicating that the degrading activity is u-PA mediated and plasmin dependent. Co-cultivation of a small portion of u-PA-producing cells with u-PAR-expressing cells resulted in a marked increase in degradation activity. Reduction of this potentiating effect by suramin or the **amino-terminal fragment** of u-PA, both competitive inhibitors of u-PA receptor binding, shows that this synergistic effect is due to binding of u-PA to u-PAR. u-PAR must be cell associated, as binding of u-PA to a soluble u-PAR prevented this enhancement. The capability of the PA system to degrade bone matrix in vitro, and the previously demonstrated increased

expression of u-PA and u-PAR in synovial tissue of patients with RA, further support a role for the PA system in the development of bone erosions.

L17 ANSWER 43 OF 109 MEDLINE on STN

97228079. PubMed ID: 9115983. Interaction of **urokinase**-type plasminogen activator with its receptor rapidly induces activation of glucose transporters. Anichini E; Zamperini A; Chevanne M; Caldini R; Pucci M; Fibbi G; Del Rosso M. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Biochemistry, **(1997 Mar 18)** 36 (11) 3076-83. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The interaction of **urokinase**-type plasminogen activator (u-PA) or of u-PA **amino-terminal fragment** (u-PA-**ATF**) with the cell surface receptor (u-PAR) was found to stimulate an increase of glucose uptake in many cell lines, ranging from normal and transformed human fibroblasts, mouse fibroblasts transfected with human u-PAR, and cells of epidermal origin. Such increase of glucose uptake reached a peak within 5-10 min, depending on the cell line, and occurred through the facilitative glucose transporters (GLUTs), since it was inhibited by cytochalasin B. Each cell line showed a specific mosaic of glucose transporter isoforms, GLUT2 being the most widespread and GLUT1 the most abundant, when present. u-PAR stimulation was followed by translocation of GLUT1 from the microsomal to the membrane compartment, as shown by both immunoblotting and immunofluorescence of sonicated plasma membrane sheets and by activation of GLUT2 on the cell surface. Both translocation and activation resulted inhibitable by protein-tyrosine kinase inhibitors and independent of downregulation of protein kinase C (PKC). The increase of intracellular glucose was followed by neosynthesis of diacylglycerol (DAG) from glucose, as previously shown. Such neosynthesis was completely inhibited by impairment of facilitative GLUT transport by cytochalasin B. DAG neosynthesis was followed by activation of PKC, whose activity translocated into the intracellular compartment (PKM), where it probably phosphorylates substrates required for u-PAR-dependent chemotaxis. Our data show that u-PAR-mediated signal transduction, related with u-PA-induced chemotaxis, involves activation of tyrosine kinase-dependent glucose transporters, leading to increased de novo DAG synthesis from glucose, eventually resulting in activation of PKC.

L17 ANSWER 44 OF 109 MEDLINE on STN

97218571. PubMed ID: 9066008. The **urokinase**-receptor (CD87) is expressed in cells of the megakaryoblastic lineage. Wohn K D; Kanse S M; Deutsch V; Schmidt T; Eldor A; Preissner K T. (Haemostasis Research Unit, Kerckhoff-Klinik, MPI, Bad Nauheim, Germany.) Thrombosis and haemostasis, **(1997 Mar)** 77 (3) 540-7. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Megakaryocytopoiesis is governed in the bone marrow microenvironment by cellular interactions that include various adhesion receptor systems and pericellular proteolysis for proper regulation of cell motility and differentiation. In order to define the role of cell surface molecules required for these processes, we searched for protease receptors on these cells. In an in vitro system utilizing different cell lines of the megakaryoblastic lineage (MEG-01, Dami), low level surface expression of the **urokinase** (uPA) receptor was noted. Following stimulation with phorbolester (PMA), a 3-6 fold higher expression of uPA receptor over a period of up to 5 days could be observed by fluorescent activated cell-sorting as well as by direct ligand-binding of **amino-terminal fragment** of uPA or vitronectin. Together with elevated expression of alpha IIb beta 3-integrin (glycoprotein IIb/IIIa complex), double immuno-fluorescence staining of stimulated cells confirmed the increased cell surface localization of uPA receptor. Semi-quantitative RT-PCR, ligand blot analysis and measurement of cell-bound proteolytic activity revealed a differentiation-dependent upregulation of the uPA receptor

expression in megakaryoblastic cell lines as in monocytic cells. Due to its glycolipid anchorage, incubation with phosphatidylinositol-specific phospholipase C reduced uPA receptor-mediated ligand binding by about 60%, uPA receptor mRNA was expressed in cultured megakaryocytes derived from bone marrow, whereas no uPA receptor mRNA was detectable in platelets. These results indicate a differentiation-dependent increase in the expression of uPA receptor in megakaryoblastic cells. The characteristics of surface expression and functionality of the receptor on megakaryocytic cells may influence their maturation by regulating cellular communication in the bone marrow micro-environment.

L17 ANSWER 45 OF 109 MEDLINE on STN

97185974. PubMed ID: 9033655. Plasminogen activators play an essential role in extracellular-matrix invasion by lymphoblastic T cells. Reiter L S; Spertini O; Kruithof E K. (Department of Medicine, University Hospital Lausanne, Switzerland.) International journal of cancer. Journal international du cancer, **(1997 Feb 7)** 70 (4) 461-6. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Involvement of extravascular sites, in particular infiltration of the central nervous system, is a frequent complication of T-lymphoblastic leukemia and contributes to leukemia-associated morbidity. In this report, we studied the contribution of plasminogen activators to the invasive properties of 7 human T-cell lines in a model of transmigration through an extracellular matrix. The T-cell lines were found to express either **urokinase** (u-PA) and high levels of u-PA receptor or tissue-type plasminogen activator (t-PA) and low levels of u-PA receptor. The rate of transmigration was consistently higher for u-PA-expressing cells than for t-PA-expressing cells. PA-inhibitor type 1 (PAI-1) was detected in the conditioned medium of one cell line and PAI-2 was detected in cell extracts from 6 lines. The transmigration of 6 out of 7 cell lines was inhibited by trasylol, an inhibitor of plasmin, by an excess of exogenous PAI-1 or PAI-2, and by antibodies to the particular PA type expressed by the cells. Partial inhibition of transmigration by the **amino-terminal fragment** of u-PA implies that the u-PA receptor contributes to transmigration. Thus, the transmigration of T-leukemia cells through a barrier of extracellular matrix requires PA-dependent proteolysis, which can be provided either by u-PA or t-PA. Specific inhibition of the PA system could provide a means to inhibit tissue invasion by T lymphoblastic cells.

L17 ANSWER 46 OF 109 MEDLINE on STN

97184198. PubMed ID: 9030610. Soluble human **urokinase** receptor is composed of two active units. Higazi A A; Mazar A; Wang J; Quan N; Griffin R; Reilly R; Henkin J; Cines D B. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) Journal of biological chemistry, **(1997 Feb 21)** 272 (8) 5348-53. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The mechanism by which single-chain **urokinase** (scuPA) binds to its receptor (uPAR) is incompletely understood. We report that a fragment comprising the first domain of recombinant soluble uPAR (sDI) as well as a fragment comprising the remaining domains (sDII-DIII) competes with the binding of recombinant full-length soluble uPAR (suPAR) to scuPA with an IC50 = 253 nM and an IC50 = 1569, respectively. sDII-III binds directly to scuPA with Kd = 238 nM. Binding of scuPA to each fragment also induces the expression of plasminogen activator activity. sDI and sDII-DIII (200 nM each) induced activity equal to 66 and 36% of the maximum activity induced by full-length suPAR (5 nM), respectively. Each fragment also stimulates the binding of scuPA to cells lacking endogenous uPAR. Although scuPA binds to sDI and to sDII-DIII through its **amino-terminal fragment**, the fragments act synergistically to inhibit the binding of suPAR and to stimulate plasminogen activator activity. Furthermore, sDII-DIII retards the velocity and alters the

pattern of cleavage of sDI by chymotrypsin. These results suggest that binding of scuPA to more than one epitope in suPAR is required for its optimal activation and association with cell membranes.

L17 ANSWER 47 OF 109 MEDLINE on STN

97022742. PubMed ID: 8869102. Immunoassays (ELISA) of **urokinase**-type plasminogen activator (uPA): report of an EORTC/BIOMED-1 workshop. Benraad T J; Geurts-Moespot J; Grondahl-Hansen J; Schmitt M; Heuvel J J; de Witte J H; Foekens J A; Leake R E; Brunner N; Sweep C G. (532 Department of Experimental and Chemical Endocrinology, University of Nijmegen, Netherlands.) European journal of cancer (Oxford, England : 1990), **(1996 Jul)** 32A (8) 1371-81. Ref: 28. Journal code: 9005373. ISSN: 0959-8049. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The **urokinase**-type plasminogen activator (uPA) is considered to play a key role in the process of invasion and metastasis. In several independent studies, in a variety of cancer types (e.g. of the breast, colon, stomach, lung, ovary), high antigen levels of uPA in tumour extracts have been associated with rapid disease progression. In these studies, different sets of antibodies and standards (often as commercially available uPA ELISA kits) have been used. The standards provided with the different uPA ELISA kits are different from each other in both composition and source. In addition, the different uPA ELISA kits use antibodies which differ in specificity and affinity for the various forms of uPA including pro-uPA, **HMW-uPA**, LMW-uPA, the aminoterminal fragment (**ATF**) and complexes with inhibitors (PAI-1 and PAI-2) and the receptor (uPAR). Further, the composition of tumour tissue extraction buffers differ significantly among the published studies. Thus, it is not surprising that the ranges of cytosolic uPA levels reported differ considerably even when measured within the same tumour type. These discrepancies led the EORTC Receptor and Biomarker Study Group, in conjunction with the BIOMED-1 consortium on 'Clinical Relevance of Proteases in Tumour Invasion and Metastasis', to organise a workshop to study the characteristics associated with six different uPA immunoassays (ELISA) used in clinical studies reported in the literature. Although the absolute uPA antigen values measured with the respective uPA ELISA kits differed, high correlations were obtained for any two of the four uPA ELISA kits finally applied to sets of breast cancer cytosol preparations. The preparations used at present as standards in the various uPA ELISA kits are not representative of actual human breast cancer cytosols. Thus absolute standardisation is only possible by using a common reference sample (breast cancer cytosol) and similarly composed ELISA uPA kits. Then it will be possible to generate comparable data on clinical tissue as well as to check for batch-to-batch variations within particular ELISA kits.

L17 ANSWER 48 OF 109 MEDLINE on STN

97010891. PubMed ID: 8857924. Contrasting effects of plasminogen activators, **urokinase** receptor, and LDL receptor-related protein on smooth muscle cell migration and invasion. Okada S S; Grobmyer S R; Barnathan E S. (University of Pennsylvania School of Medicine, Philadelphia 19104-6060, USA.) Arteriosclerosis, thrombosis, and vascular biology, **(1996 Oct)** 16 (10) 1269-76. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Smooth muscle cell (SMC) migration is an early response to vascular injury and contributes to the development of intimal thickening. Upregulation of several components of the plasminogen activator (PA) system has been documented after vascular injury. Utilizing a Transwell filter assay system and human umbilical vein SMCs, we sought to define the role of four different PA system components on SMC migration and matrix invasion: (1) PAs, (2) plasmin, (3) PA receptors, and (4) PA clearance receptors (ie, low density lipoprotein receptor-related protein [LRP]). Addition of active two-chain **urokinase**-type PA (UPA) stimulated random migration (192 +/- 30% of control, 0.36 nmol/L, $P < .001$). The stimulation was

inhibited by pretreatment with diisopropylfluorophosphate, PA inhibitor type 1 (PAI-1), or aprotinin, a plasmin inhibitor. Augmented migration was also observed with either low-molecular-weight UPA or the **amino terminal fragment** of UPA (**ATF**), with the effects being additive. Stimulation by **ATF** alone, however, was not inhibited by aprotinin. The stimulatory effect was not specific for UPA, in that tissue-type PA (TPA) also increased migration (169 +/- 9% of control, 10 nmol/L, P < .001); the augmentation was inhibited by pretreatment with DFP, PAI-1, or aprotinin and was additive to the UPA effect. Antibodies to the UPA receptor but not 5'-nucleotidase (another glycosylphosphatidylinositol-anchored cell surface protein) inhibited baseline and UPA-stimulated migration. Similarly, both UPA and TPA stimulated invasion of a collagen gel; this augmentation was inhibited by aprotinin, whereas antibodies to the UPA receptor reduced baseline invasion. Finally, we tested whether inhibition of LRP function, which mediates internalization of PA/inhibitor complexes, affected either process. Both antibodies to LRP and recombinant receptor associated protein, a known inhibitor of ligand binding to the LRP, significantly inhibited migration but did not affect collagen gel invasion. These data demonstrate the ability of several components of the PA system to modulate SMC migration and invasion in vitro via plasmin-dependent and -independent mechanisms.

L17 ANSWER 49 OF 109 MEDLINE on STN

96404689. PubMed ID: 8808830. Mechanism of tumor cell-induced extracellular matrix degradation--inhibition of cell-surface proteolytic activity might have a therapeutic effect on tumor cell invasion and metastasis. Kobayashi H. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine.) Nippon Sanka Fujinka Gakkai zasshi, **(1996 Aug)** 48 (8) 623-32. Ref: 14. Journal code: 7505749. ISSN: 0300-9165. Pub. country: Japan. Language: Japanese.

AB Tumor cells produce **urokinase**-type plasminogen activator (uPA) in an enzymatically inactive proenzyme form (pro-uPA). Secreted pro-uPA can immediately bind to the specific uPA receptors (uPAR) on tumor cell surface with high affinity. The uPAR specifically recognizes enzymatically inactive pro-uPA and active high molecular weight-uPA (**HMW-uPA**) by their growth factor-like terminal domain. uPAR is a glycoprotein of approximately 55 kDa; the affinity for uPA is high (0.2 nM) and the rate of dissociation is low. Receptor-bound uPA catalizes the formation of plasmin on the cell surface to generate the proteolytic cascade that contributes to the breakdown of basement membrane and extracellular matrix. The plasma membrane uPAR has attracted considerable attention because of its role in migration and tissue invasion by mononuclear phagocytes and malignant cells. In some cell types uPAR localizes uPA to cell-cell and cell-substratum contact sites, providing the possibility of a directional proteolysis that may be involved in cell migration and invasion. Recently it has been reported that competitive displacement of uPA from uPAR resulted in decreased proteolysis, suggesting that the cell surface is the preferred site for uPA-mediated protein degradation. Various very different approaches to interfere with the expression or reactivity of uPA or uPAR at the gene or protein level were successfully tested including antisense oligonucleotides, antibodies, inhibitors and recombinant or synthetic uPA and uPAR analogues. Recently we have reported that a highly purified urinary trypsin inhibitor (UTI) efficiently inhibits soluble and tumor cell-surface receptor-bound plasmin. UTI inhibits not only tumor cell invasion in an in vitro assay but also production of experimental and spontaneous lung metastasis in an in vivo mouse model. The anti-invasive effect is dependent on the anti-plasmin activity of UTI. UTI peptide, which inhibits plasmin activity, synthesized by an automated peptide synthesizer showed mouse 3LL cell invasion inhibitory activity. UTI and the effective peptide inhibited tumor cell invasion through Matrigel. UTI did not inhibit tumor cell proliferation or the binding of the cells to Matrigel. Also, UTI did not inhibit chemotactic migration of tumor cells to fibronectin. It is

likely that UTI acts as a protease inhibitor. We attempted to synthesize conjugates between **ATF** and UTI. Thus, conjugating a physiological plasmin inhibitor to **ATF** might target it to reduce cell-associated proteolytic activity to the close environment of the uPAR-expressing tumor cell surface and subsequently may effectively inhibit tumor cell invasion and metastasis, because the cell surface uPAR might be a critical component of the metastatic machinery. A method of conjugation of the UTI domain II (HI-8), to the receptor-binding **amino-terminal fragment** (**ATF**) of uPA has been developed utilizing the heterobifunctional cross-linking reagent, N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). The conjugate retained its protease inhibiting activity and showed a binding reactivity to uPAR on the surface of tumor cells. We have shown that the conjugate exhibits plasmin inhibition to the close environment of the cell surface and subsequently inhibits the tumor cell invasion through Matrigel in an in vitro invasion assay. In order to extend our idea, we attempt to produce a novel hybrid molecule consisting of the **ATF** of uPA placed at the N-terminus of UTI domain II (HI-8) by protein engineering techniques. Exogenously applied ATFHI hybrid protein can immediately bind to the specific uPAR on cell surfaces with high affinity. The receptor-bound hybrid protein focuses the protease-inhibiting activity to the tumor cell surface. This is effectively a bifunctional molecule which, in addition to inhibiting trypsin and plasmin activities directly, is able toblock unoccupied uPAR, thereby preventing localization of uPA activity.

L17 ANSWER 50 OF 109 MEDLINE on STN

96394362. PubMed ID: 8798468. Domain interplay in the **urokinase** receptor. Requirement for the third domain in high affinity ligand binding and demonstration of ligand contact sites in distinct receptor domains. Behrendt N; Ronne E; Dano K. (Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, Building 7. 2, DK-2100 Copenhagen O, Denmark.) Journal of biological chemistry, **(1996 Sep 13)** 271 (37) 22885-94. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The **urokinase** plasminogen activator receptor (uPAR) is a membrane protein comprised of three extracellular domains. In order to study the importance of this domain organization in the ligand-binding process of the receptor we subjected a recombinant, soluble uPAR (suPAR) to specific proteolytic cleavages leading to liberation of single domains. Treatment of the receptor with pepsin resulted in cleavage between residues 183 and 184, thus separating the third domain (D3) from the rest of the molecule, which was left as an intact fragment (D(1 + 2)). D(1 + 2) proved capable of ligand binding as shown by chemical cross-linking, but quantitative binding/competition studies showed that the apparent ligand affinity was 100- to 1000-fold lower than that of the intact suPAR. This loss of affinity was comparable with the loss found after cleavage between the first domain (D1) and D(2 + 3), using chymotrypsin. This result shows that in addition to D1, which has an established function in ligand binding (Behrendt, N., Ploug, M., Patthy, L., Houen, G., Blasi, F., and Dano, K. (1991) J. Biol. Chem. 266, 7842-7847), D3 has an important role in governing a high affinity in the intact receptor. Real-time biomolecular interaction analysis revealed that the decrease in affinity was caused mostly by an increased dissociation rate of the ligand complex of D(1 + 2). Zero length cross-linking, using carbodiimide-induced, direct condensation, was used to identify regions within suPAR engaged in molecular ligand contact. The purified suPAR was cross-linked to the radiolabeled **amino-terminal fragment** (**ATF**) of **urokinase**, followed by cleavage with chymotrypsin. In accordance with the cleavage pattern found for the uncomplexed receptor, this treatment led to cleavage between D1 and D(2 + 3). Analysis of the radiolabeled fragments revealed the expected ligand labeling of D1 but a clear labeling of D(2 + 3) was also found, indicating that this part of the molecule is also situated in close contact with **ATF** in the receptor-ligand complex. The latter

contact site may contribute to the role of molecular regions outside D1 in high affinity binding.

L17 ANSWER 51 OF 109 MEDLINE on STN

96273549. PubMed ID: 8695276. In vitro anti-proliferative and anti-invasive role of aminoterminal fragment of **urokinase**-type plasminogen activator on 8701-BC breast cancer cells. Luparello C; Del Rosso M. (Dipartimento di Biologia Cellulare e dello Sviluppo, Universita, Palermo, Italy.) European journal of cancer (Oxford, England : 1990), **(1996 Apr)** 32A (4) 702-7. Journal code: 9005373. ISSN: 0959-8049. Pub. country: ENGLAND: United Kingdom. Language: English.

AB 8701-BC cells, derived from a primary carcinoma of the breast, constitutively express mRNA for **urokinase**-type plasminogen activator (uPA). In this paper, we demonstrated the presence of uPA in the conditioned medium, and of uPA-receptor (uPAR) on the cell surface of 8701-BC cells, which therefore have the potential for an autocrine mechanism of uPA-mediated stimulation. We examined whether exogenous addition of either intact uPA, or its **amino-terminal fragment** (uPA-**ATF**), which lacks catalytic activity but retains the uPAR binding site and a growth factor-like domain, or immunoneutralisation of endogenous uPA-uPAR interactions could exert any effect on the proliferative and invasive behaviour of 8701-BC cells. The data demonstrate that, while uPA promotes growth and invasion of 8701-BC cells, its effect reversed by blocking uPA-uPAR interactions, uPA-**ATF** not only fails to impart growth factor-like signals, but also restrains cell invasion in vitro. In the light of these and other data, an active participation of **ATF** in the complex cell-ECM network of interactions underlying cancer progression can be postulated. In addition, it appears worth considering the possibility of testing the effect of this uPA fragment in vivo for the therapy of breast (and possibly other) human invasive carcinomas.

L17 ANSWER 52 OF 109 MEDLINE on STN

96235241. PubMed ID: 8647121. Systematic mutational analysis of the receptor-binding region of the human **urokinase**-type plasminogen activator. Magdolen V; Rettenberger P; Koppitz M; Goretzki L; Kessler H; Weidle U H; Konig B; Graeff H; Schmitt M; Wilhelm O. (Frauenklinik der Technischen Universitat Munchen, Germany.) European journal of biochemistry / FEBS, **(1996 May 1)** 237 (3) 743-51. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The **amino-terminal fragment** of human uPA (**ATF**; amino acids 1-135), which contains the binding site for the uPA receptor (uPAR, CD87) was expressed in the yeast Saccharomyces cerevisiae. Recombinant yeast **ATF**, modified and extended by an amino-terminal in-frame insertion of a His6 tract, was purified from total protein extracts by nickel chelate affinity chromatography and shown to be functionally active since it efficiently competes with uPA for binding to cell-surface-associated uPAR. The **ATF** expression plasmid served as a template for the construction of a series of site-directed mutants in order to define those amino acids that are important for binding to uPAR. All mutant **ATF** proteins but one (deletion of Ser26) were expressed in a stable form (about 20-30 ng/mg total protein) and the binding capacity of each mutant was tested by a uPA-ligand binding assay employing recombinant uPAR immobilized to a microtiter plate. Each of the 11 amino acids of loop B of the binding region of uPA (amino acids 20-30) were individually substituted with alanine. Lys23, Tyr24, Phe25, IIe28, and Trp30 were important determinants for uPAR binding. A systematic alanine scan was also performed with chemically synthesized linear peptides spanning amino acids 14-32 of **ATF**. Comparable results to those with the yeast **ATF** mutants were obtained. In a different set of experiments, those amino acids of the uPAR-binding region of uPA that are only conserved between man and baboon but not in other species were altered: whereas substitution of

Thr18 by alanine or Asn32 by serine had hardly any effect, replacement of Asn22 by tyrosine and Trp30 by arginine (both positions are strictly conserved in other mammals) led to **ATF** variants incapable of interacting with human uPAR. Deletion of either Val20, Ser21, Lys23, His29 or Val20 plus Ser21, respectively, also generated non-reactive **ATF** mutants. Finally, Lys23 in **ATF** was substituted with certain amino acids: whereas the replacement of Lys23 by alanine, histidine or glutamine generated **ATF** variants with moderate uPAR-binding activity, the introduction of a negatively charged amino acid (exchange of Lys23 by glutamic acid) completely abolished uPAR-binding activity. The results presented for the **ATF** mutants and uPA-derived peptides may provide clues necessary to establish the nature of the physical interaction of uPA with its receptor and may help to develop uPA-derived peptide analogues as potential therapeutic agents to block tumor cell-associated uPA/uPAR interaction.

L17 ANSWER 53 OF 109 MEDLINE on STN

96219970. PubMed ID: 8639894. Evidence for a novel binding protein to **urokinase**-type plasminogen activator in platelet membranes. Jiang Y; Pannell R; Liu J N; Gurewich V. (Institute for the Prevention of Cardiovascular Disease, Deaconess Hospital, Harvard Medical School, Boston, MA, USA.) Blood, **(1996 Apr 1)** 87 (7) 2775-81. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Endogenous **urokinase**-type plasminogen activator (u-PA) has been identified in platelet membrane, and platelets have been shown to take up exogenous high molecular weight u-PA from the ambient medium. In this report, the mechanism of the association of u-PA with platelets was investigated using recombinant, single chain u-PA. When gel filtered human platelets were incubated with radiolabeled u-PA, the u-PA was found to specifically and saturably bind to the resting platelets in a dose-dependent manner. Unlabeled u-PA and the **amino terminal fragment** of u-PA inhibited 125I-u-PA binding to platelets with a mean IC50 of 65 and 58 nmol/L, respectively. A single saturable binding site in intact resting platelets was found with a mean kd of 43 +/- 25 nmol/L and 2263 +/- 809 sites per platelet. In contrast to resting platelets, 125I-u-PA did not bind to thrombin-induced platelets. Western blotting studies, using a monoclonal or a polyclonal antibody specific for the u-PA cell-surface receptor (u- PAR), failed to show evidence of u-PAR in resting platelets, whereas, u-PAR was found at approximately 54 and approximately 48 kD on U937 monocytes, which served as a positive control. Ligand blotting of platelet membrane and of U937 cell proteins with 125I-u-PA revealed a u-PA binding protein of approximately 70 kD in the platelets and one of approximately 54 kD in the U937 cells. Complexion of u-PA with a platelet membrane protein was also shown by gel filtration of a mixture of u-PA and platelet membrane proteins. A u-PA complex was further shown by enzyme-linked immunosorbent assay when microtiter plates were coated with platelet membrane proteins, and this complex formation was shown to be dose-dependent and saturable with an apparent kd of 17 nmol/L. It was concluded that platelet membrane contains a specific, high affinity u-PA-binding protein that is distinct from u-PAR.

L17 ANSWER 54 OF 109 MEDLINE on STN

96203075. PubMed ID: 8612581. Proteolytic cleavage of the **urokinase** receptor substitutes for the agonist-induced chemotactic effect. Resnati M; Guttinger M; Valcamonica S; Sidenius N; Blasi F; Fazioli F. (Department of Biology and Biotechnology, San Raffaele Scientific Institute, Milano, Italy.) EMBO journal, **(1996 Apr 1)** 15 (7) 1572-82. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Physiological concentrations of **urokinase** plasminogen activator (uPA) stimulated a chemotactic response in human monocytic THP-1 through binding to the **urokinase** receptor (uPAR). The effect did not require the protease moiety of uPA, as stimulation was achieved also with the N-terminal fragment (**ATF**), while the 33 kDa low molecular weight uPA was

ineffective. Co-immunoprecipitation experiments showed association of uPAR with intracellular kinase(s), as demonstrated by in vitro kinase assays. Use of specific antibodies identified p56/p59hck as a kinase associated with uPAR in THP-1 cell extracts. Upon addition of **ATF**, p56/p59hck activity was stimulated within 2 min and returned to normal after 30 min. Since uPAR lacks an intracellular domain capable of interacting with intracellular kinase, activation of p56/p59hck must require a transmembrane adaptor. Evidence for this was strongly supported by the finding that a soluble form of uPAR (suPAR) was capable of inducing chemotaxis not only in THP-1 cells but also in cells lacking endogenous uPAR (IC50, 5 pM). However, activity of suPAR require chymotrypsin cleavage between the N-terminal domain D1 and D2 + D3. Chymotrypsin-cleaved suPAR also induced activation of p56/p59hck in THP-1 cells, with a time course comparable with **ATF**. Our data show that uPA-induced signal transduction takes place via uPAR, involves activation of intracellular tyrosine kinase(s) and requires an as yet undefined adaptor capable of connecting the extracellular ligand binding uPAR to intracellular transducer(s).

L17 ANSWER 55 OF 109 MEDLINE on STN

96198033. PubMed ID: 8612711. The **urokinase** receptor is a major vitronectin-binding protein on endothelial cells. Kanse S M; Kost C; Wilhelm O G; Andreasen P A; Preissner K T. (Haemostasis Research Unit, Kerckhoff-Klinik, Bad Nauheim, Germany.) Experimental cell research, **(1996 May 1)** 224 (2) 344-53. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We have previously demonstrated that vitronectin (VN), a morphoregulatory protein in the vessel wall, is internalized and translocated to the subendothelial matrix by an integrin-independent mechanism (J. Histochem. Cytochem. 41, 1823-1832, 1993). The cell surface component which mediates the initial contact of VN with endothelial cells is defined here. The specific binding of VN to endothelial cells demonstrated the following properties: a threefold increase after phorbol ester treatment; 85% inhibition by pretreatment of cells with phosphatidylinositol-phospholipase C to release glycolipid-anchored surface proteins; a 90% inhibition by **urokinase** (u-PA) receptor blocking antibody. u-PA increased VN binding to cells due to an eightfold increase in the affinity of VN for the u-PA receptor. Structure-function studies showed that the **amino-terminal fragment** of u-PA, devoid of any proteolytic activity, mediated this effect. Active plasminogen activator inhibitor-1 (PAI-1), but not inactivated PAI-1, inhibited VN binding to cells and displaced VN that was prebound to endothelial cell monolayers. Similarly, VN binding to purified (immobilized) u-PA receptor, but not to integrin, was enhanced by u-PA and inhibited by PAI-1. Hence, the binding of soluble VN to endothelial cell surfaces is mediated by the u-PA receptor, and the relative concentrations of u-PA and PAI-1 are able to regulate the strength of this interaction. Endothelial cell adhesion to immobilized VN was found to be integrin-mediated without any involvement of the VN-uPA-receptor system. Hence, the interaction of VN with the u-PA receptor may be involved in the regulation of cellular processes necessary for endothelial cell invasion and migration at VN-rich extracellular matrix sites.

L17 ANSWER 56 OF 109 MEDLINE on STN

96193900. PubMed ID: 8641412. Removal of domain D2 or D3 of the human **urokinase** receptor does not affect ligand affinity. Riittinen L; Limongi P; Crippa M P; Conese M; Hernandez-Marrero L; Fazioli F; Blasi F. (Department of Biology and Biotechnology (DIBIT), San Raffaele Scientific Institute, Milan, Italy.) FEBS letters, **(1996 Feb 26)** 381 (1-2) 1-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The main ligand-binding determinant of the human **urokinase** receptor (uPAR) is located in the amino terminal domain D1, but when isolated this

domain presents a 1500 fold lower affinity than the intact three-domain uPAR (D1D2D3). uPAR mutants missing either domain 2 (D1HD3) or domain 3 (D1D2) were expressed in murine LB6 cells and showed to be properly GPI-anchored to the cell surface. Binding assays with [125I]**ATF** demonstrated that these mutants possessed a normal (D1D2) or slightly reduced (D1HD3) affinity, indicating that a high ligand-affinity may be achieved by a combination of D1 with domain D2 or D3.

L17 ANSWER 57 OF 109 MEDLINE on STN

96181660. PubMed ID: 8603739. Blockage of **urokinase** receptor reduces in vitro the motility and the deformability of endothelial cells. Lu H; Mabilat C; Yeh P; Guitton J D; Li H; Pouchelet M; Shoevaert D; Legrand Y; Soria J; Soria C. (INSERM U353, Hopital St. Louis, Paris, France.) FEBS letters, **(1996 Feb 12)** 380 (1-2) 21-4. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The binding of **urokinase** (u-PA) to its cell surface receptor (u-PAR) is critical for tumor cell invasion. Here, we report that the distribution of this binding by a u-PAR antagonist **ATF**-HSA inhibits in vitro the motility of endothelial cells in a dose-dependent manner. This inhibition was also observed when the cells were first stimulated with potent angiogenic factors, including bFGF or VEGF. [3H]thymidine incorporation assay demonstrated that **ATF**-HSA did not affect the cell proliferation. **ATF**-HSA was more potent than plasmin inhibitors, suggesting that it exerts its effects not solely by inhibiting the remodeling of the extracellular matrix. In fact, analysis of the cell shape change during migration revealed for the first time that its effect is related to a decrease in cell deformability. These results suggest that u-PAR antagonist may be a new approach to control angiogenesis.

L17 ANSWER 58 OF 109 MEDLINE on STN

96176310. PubMed ID: 8601593. Cooperative effect of TNFalpha, bFGF, and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of **urokinase** activity. Koolwijk P; van Erck M G; de Vree W J; Vermeer M A; Weich H A; Hanemaaijer R; van Hinsbergh V W. (Gaubius Laboratory TNO-PG, Leiden, The Netherlands.) Journal of cell biology, **(1996 Mar)** 132 (6) 1177-88. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB In angiogenesis associated with tissue repair and disease, fibrin and inflammatory mediators are often involved. We have used three-dimensional fibrin matrices to investigate the humoral requirements of human microvascular endothelial cells (hMVEC) to form capillary-like tubular structures. bFGF and VEGF165 were unable to induce tubular structures by themselves. Simultaneous addition of one or both of these factors with TNFalpha induced outgrowth of tubules, the effect being the strongest when bFGF, VEGF165, and TNFalpha were added simultaneously. Exogenously added u-PA, but not its nonproteolytic **amino-terminal fragment**, could replace TNFalpha, suggesting that TNFalpha-induced u-PA synthesis was involved. Soluble u-PA receptor (u-PAR) or antibodies that inhibited u-PA activity prevented the formation of tubular structures by 59-99%. epsilon-ACA and trasylol which inhibit the formation and activity of plasmin reduced the extent of tube formation by 71-95%. TNFalpha or u-PA did not induce tubular structures without additional growth factors. bFGF and VEGF165 enhanced of the u-PAR by 72 and 46%, but TNFalpha itself also increased u-PAR in hMVEC by 30%. Induction of mitogenesis was not the major contribution of bFGF and VEGF165 because the cell number did not change significantly in the presence of TNFalpha, and tyrphostin A47, which inhibited mitosis completely, reduced the formation of tubular structures only by 28-36%. These data show that induction of cell-bound u-PA activity by the cytokine TNFalpha is required in addition to the angiogenic factors VEGF165 and/or bFGF to induce in vitro formation of capillary-like structures by hMVEC in fibrin matrices. These data may provide insight in the mechanism of angiogenesis as occurs in pathological conditions.

L17 ANSWER 59 OF 109 MEDLINE on STN
96145724. PubMed ID: 8590627. A competitive chromogenic assay to study the functional interaction of **urokinase**-type plasminogen activator with its receptor. Rettenberger P; Wilhelm O; Oi H; Weidle U H; Goretzki L; Koppitz M; Lottspeich F; Konig B; Pessara U; Kramer M D; +. (Frauenklinik, Technischen Universitat Munchen, Germany.) Biological chemistry Hoppe-Seyler, **(1995 Oct)** 376 (10) 587-94. Journal code: 8503054. ISSN: 0177-3593. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) converts plasminogen to plasmin which degrades various extracellular matrix components. uPA is focused to the cell surface via binding to a specific receptor (uPAR, also termed CD87). uPAR-bound uPA mediates pericellular proteolysis in a variety of biological processes, e.g. cell migration, tissue remodeling and tumor invasion. We have developed a competitive microtiter plate-based chromogenic assay which allows the analysis of uPA/uPAR interaction. The plates are coated with recombinant uPAR expressed in Chinese hamster ovary (CHO) cells. Proteolytically active uPA (**HMW-uPA**) is added to the microtiter plate-attached uPAR. The amount of receptor-bound uPA is then determined indirectly via addition of plasminogen, which is activated to plasmin, followed by cleavage of a plasmin-specific chromogenic substrate. Substances interfering with binding of **HMW-uPA** to uPAR diminish the generation of plasmin, as indicated by a reduction of cleaved chromogenic substrate. This assay was used to analyze the inhibitory capacity of a variety of proteins and peptides, respectively, on the uPA/uPAR interaction: i) uPAR and uPAR-variants expressed in CHO cells, yeast or E. coli, ii) the aminoterminal fragment (**ATF**) of human uPA or yeast recombinant pro-uPA, iii) synthetic peptides derived from the sequence of the uPAR-binding region of uPA, and iv) antibodies directed against uPAR. This assay may be helpful in identifying uPA and uPAR analogues or antagonists which efficiently block uPA/uPAR interaction.

L17 ANSWER 60 OF 109 MEDLINE on STN
96107172. PubMed ID: 8530448. **Urokinase**-type plasminogen activator-induced monocyte adhesion requires a carboxyl-terminal lysine and cAMP-dependent signal transduction. Li C; Liu J N; Gurewich V. (Vascular Research Laboratory, Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215, USA.) Journal of biological chemistry, **(1995 Dec 22)** 270 (51) 30282-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (u-PA) or its **amino-terminal fragment** (**ATF**) containing the u-PA receptor (u-PAR) binding domain, is known to promote monocyte adhesion. In the present study, U937 monocyte adhesion to a plastic surface was used to investigate the mechanism of its promotion by u-PA and **ATF**. Adhesion was found to be inhibited by cycloheximide or actinomycin D, implicating protein synthesis and gene expression in u-PA-induced monocyte adhesion. Adhesion was prevented by 2'-deoxyadenosine 3'-monophosphate, indicating that a cAMP-dependent pathway of signal transduction was involved. This concept was supported by the complementary finding that u-PA-induced adhesion was greatly promoted by forskolin, cholera toxin, or 8-bromo-cAMP, which by themselves induced little adhesion. Furthermore, similar to many other cAMP-dependent activities, cGMP diminished u-PA-induced adhesion. When u-PA or **ATF** was treated with immobilized carboxypeptidase B, its proadhesive effect was abolished, implicating the involvement of carboxyl-terminal lysine residues (Lys158 on u-PA and Lys135 on **ATF**). Moreover, when a carboxyl-terminal lysine analog was added, the proadhesive effect of carboxypeptidase B-treated u-PA or **ATF** was restored. In conclusion, the present study indicates that u-PA- or **ATF**-induced monocyte adhesion involves cAMP-dependent signal transduction, which is triggered by u-PAR binding. It is also critically

dependent on the presence of a carboxyl-terminal lysine.

L17 ANSWER 61 OF 109 MEDLINE on STN
96049541. PubMed ID: 7586807. Increase of a **urokinase** receptor-related low-molecular-weight molecule in colorectal adenocarcinomas. Lau H K; Kim M; Koo J; Chiu B; Murray D. (Division of Hematology and Oncology, St Michael's Hospital, Toronto, Ontario, Canada.) Clinical & experimental metastasis, **(1995 Nov)** 13 (6) 492-8. Journal code: 8409970. ISSN: 0262-0898. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Proteolytic activity is important for tumor growth and metastasis. Plasminogen and **urokinase**-type plasminogen activator (u-PA) constitute one of the most extensively studied proteolytic systems believed to participate in these processes. u-PA cleaves plasminogen to plasmin, which in turn degrades surrounding extracellular matrix and allows tumor cells to migrate to other areas. The specific receptor for u-PA (u-PAR) has also been implicated as an essential modulator in this pathway. Eleven paired samples of colorectal cancers and normal mucosal tissues from the same patients were removed at surgery. The tissues were homogenized and the supernatants assayed for u-PAR immunoreactivity, u-PAR antigen concentration, u-PAR binding activity and u-PA activity. Immunoblot analysis showed that a major u-PAR species of approximately 55 kDa was present in all tissues. In addition, a protein band of approximately 41 kDa, which crossreacted with anti-u-PAR antibodies, was also found in the tumors. This protein band was either absent, or present in relatively small amounts in the normal colorectal tissues. Cross-linking experiments showed that the approximately 55 kDa band only, and not the approximately 41 kDa band, was able to bind either single chain **urokinase**-type plasminogen activator (scu-PA) or the **amino terminal fragment** of **urokinase** (**ATF**). The tumor samples also exhibited highly elevated u-PA activity and u-PAR antigen relative to the corresponding normal tissues. Elevated u-PA activity appeared to correlate with elevated u-PAR antigen in colorectal cancers, but not in the normal tissues. These increases were also associated with increase of the u-PAR-related, low-molecular-weight protein in the tumor samples. The measurement of u-PAR and the u-PAR-related protein, in addition to u-PA activity, could have diagnostic or prognostic value in this type of cancer.

L17 ANSWER 62 OF 109 MEDLINE on STN
95349959. PubMed ID: 7624151. Heterodimerization of c-Jun with **ATF**-2 and c-Fos is required for positive and negative regulation of the human **urokinase** enhancer. De Cesare D; Vallone D; Caracciolo A; Sassone-Corsi P; Nerlov C; Verde P. (International Institute of Genetics and Biophysics, Naples, Italy.) Oncogene, **(1995 Jul 20)** 11 (2) 365-76. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Dimerization plays a pivotal role in modulating the activity of the c-Jun proto-oncogene product. Heterodimerization with activating transcription factor-2 (**ATF**-2) alters the DNA-binding specificity of c-Jun, allowing its targeting to several cAMP responsive element (CRE)-related sequences, which control a subset of AP-1-responsive genes. Here we show that a c-Jun/**ATF**-2 heterodimer binds to the AP-1 site (uPA 5'-TRE) essential for the activity of the human **urokinase** enhancer, conferring on this element several distinctive regulatory properties. The c-Jun/**ATF**-2 heterodimer was identified by binding competition assays, u.v. cross linking, and monospecific antibodies. In vitro binding studies revealed that the uPA 5'-TRE sequence is recognized by the cyclic AMP-unresponsive **ATF**-2 factor, but not by the cyclic AMP-inducible CREB. In addition, in vivo studies suggest that **ATF**-2 can mediate, at the same time, the activation of the c-Jun/**ATF**-2 site and the repression of the canonical collagenase AP-1 site. We report that heterodimerization with c-Fos does not increase the binding of c-Jun to the uPA 5'-TRE, in contrast to the increased binding at a consensus AP-1 site. Our data further suggest that c-Fos can act as a repressor of the c-Jun/**ATF**-2 binding site, revealing

an important functional difference, with respect to canonical AP-1 elements.

L17 ANSWER 63 OF 109 MEDLINE on STN

95335838. PubMed ID: 7611439. **Urokinase** receptor in human malignant mesothelioma cells: role in tumor cell mitogenesis and proteolysis. Shetty S; Kumar A; Johnson A; Pueblitz S; Idell S. (Department of Medicine, University of Texas Health Science Center at Tyler 75710, USA.) American journal of physiology, **(1995 Jun)** 268 (6 Pt 1) L972-82. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB **Urokinase** (uPA) interacts with its receptor (uPAR) to promote proteolysis and tumor migration, functions of potential importance in the pathogenesis of malignant mesothelioma. Immunohistochemistry of human malignant mesothelioma tissue and mesothelioma cells (MS-1) showed that mesothelioma cells express uPAR. We isolated uPAR from MS-1 cells by metabolic labeling and showed that it could be induced by phorbol myristate acetate (PMA), lipopolysaccharide (LPS), a transforming growth factor-beta (TGF-beta) or tumor necrosis factor-alpha (TNF-alpha). Experiments with MS-1 cells showed that uPA binding was saturable, specific, and reversible with a mean dissociation constant (Kd) of 5.4 +/- 1.1 nM. Binding was inhibited by a blocking antibody to uPAR and by the uPA **amino-terminal fragment** (**ATF**), but not by low molecular weight uPA. uPAR expression was regulated transcriptionally and translationally; antisense oligonucleotides blocked expression of uPAR protein. Plasminogen activator inhibitor-1 (PAI-1) inhibited PA activity of preformed uPA/uPAR complexes and increased cycling of the receptor from the cell surface. Stimulation of subconfluent MS-1 cells by high molecular weight or recombinant uPA, but not **ATF** or low molecular weight fragment, caused concentration-dependent incorporation of [3H]thymidine. These data indicate a novel mechanism by which malignant mesothelioma cells localize pericellular proteolysis and concurrently regulate tumor cell proliferation.

L17 ANSWER 64 OF 109 MEDLINE on STN

95329570. PubMed ID: 7605874. Studies of possible mechanisms for the effect of **urokinase** therapy in small cell carcinoma of the lung. Meehan K R; Zacharski L R; Maurer L H; Howell A L; Memoli V A; Rousseau S M; Hunt J A; Henkin J. (Department of Veterans Affairs Medical & Regional Office Center, White River Junction, Vermont 05009, USA.) Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, **(1995 Apr)** 6 (2) 105-12. Journal code: 9102551. ISSN: 0957-5235. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Urokinase**-type plasminogen activator has been administered by other investigators to patients with small cell carcinoma of the lung (SCCL) in an attempt to induce lysis of fibrin that is known to exist in the connective tissue stroma of this tumour type and that may support tumour growth. To study the fate of infused **urokinase** in this disease, a biopsy of a scalp metastasis was obtained from a patient with SCCL (entered on a phase I clinical trial of **urokinase** plus combination chemotherapy) immediately following **urokinase** infusion during the fourth course of therapy a time when this tumour mass had decreased to approximately 25% of its original size. Immunohistochemical procedures revealed abundant stromal fibrin in accord with previous observations from this laboratory. By contrast, **urokinase**, that is not a feature of small cell tumour cells, was present on the tumour cells in this specimen. **Urokinase** infusion was associated with a rapid increase in the amount of this enzyme associated with isolated peripheral blood monocytes. These results are consistent with uptake of infused **urokinase** onto monocytes and possibly tumour cells. It is postulated that substantial tumour fibrinolysis may not accompany such therapy and that **urokinase**, or its **amino terminal fragment** that bears the growth factor domain of this molecule, may bind to and alter the growth of the tumour cells.

L17 ANSWER 65 OF 109 MEDLINE on STN

95229658. PubMed ID: 7713945. Inhibitory effect of a conjugate between human **urokinase** and urinary trypsin inhibitor on tumor cell invasion in vitro. Kobayashi H; Gotoh J; Hirashima Y; Fujie M; Sugino D; Terao T. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Shizuoka, Japan.) Journal of biological chemistry, **(1995 Apr 7)** 270 (14) 8361-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Proteolytic enzymes such as **urokinase**-type plasminogen activator (uPA), plasmin, and collagenase mediate proteolysis by a variety of tumor cells. uPA secreted by tumor cells can be bound to a cell surface receptor via a growth factor-like domain within the **amino-terminal fragment** (**ATF**) of the uPA molecule with high affinity. Urinary trypsin inhibitor (UTI) efficiently inhibits the soluble and the tumor cell-surface receptor-bound plasmin and subsequently reduces tumor cell invasion and the formation of metastasis. The anti-invasive effect is dependent on the anti-plasmin activity of the UTI molecule, domain II in particular. We synthesized a conjugate between **ATF** of human uPA and a native UTI molecule or domain II of UTI (HI-8). The effect of the conjugates (**ATF**.UTI or **ATF**.HI-8) on tumor cell invasion in vitro was investigated. **ATF**.UTI and **ATF**.HI-8 bound to U937 cells in a rapid, saturable, dose-dependent, and reversible manner. A large part of receptor-bound **ATF**-UTI and **ATF**.HI-8 remains on the cell surface for at least 5 h at 37 degrees C. Inhibition of tumor cell-surface receptor-bound plasmin by **ATF**.UTI and **ATF**.HI-8 was markedly enhanced when compared with tumor cells treated either with **ATF**, UTI, or HI-8. Results of a cell invasion assay showed that **ATF**.UTI and **ATF**.HI-8 is very effective at targeting HI-8 specifically to uPA receptor-expressing tumor cells, whereas tumor cells devoid of uPA receptor may be less affected by the conjugates. Our results indicate that cell surface uPA and plasmin activity is essential to the invasive process and that the conjugates exhibit plasmin inhibition to the close environment of the cell surface and subsequently inhibit the tumor cell invasion through Matrigel in an in vitro invasion assay.

L17 ANSWER 66 OF 109 MEDLINE on STN

95213999. PubMed ID: 7699636. Control of the chondrocyte fibrinolytic balance by the drug piroxicam: relevance to the osteoarthritic process. Fibbi G; Serni U; Matucci A; Mannoni A; Pucci M; Anichini E; Del Rosso M. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Journal of rheumatology, **(1994 Dec)** 21 (12) 2322-8. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE. Since the plasminogen activator [PA/plasminogen activator inhibitor (PAI) system is believed to be involved in a breakdown of articular cartilage in osteoarthritis (OA), we studied the modulation of single components of the fibrinolytic system (**urokinase**-type plasminogen activator, u-PA; plasminogen activator inhibitor-1, PAI-1; the surface receptor for u-PA, u-PAR) in human chondrocytes in the presence of piroxicam. METHODS. The drug was added to the chondrocyte culture medium either directly or by supplementing chondrocyte cultures with synovial fluid (SF) from patients with OA treated with piroxicam. We have shown u-PAR M(r) 55000 Da on human chondrocytes in suspension culture by cross linking chondrocyte lysates with 125I-labelled **amino-terminal fragment** (**ATF**) of human u-PA, which frames the sequence that specifically interacts with u-PAR. RESULTS. Such receptors decrease upon incubation of chondrocytes with piroxicam or with SF from patients treated with piroxicam. The culture medium of treated chondrocytes showed decreased fibrinolytic activity when compared with untreated controls, while PAI activity was increased in both SF chondrocyte culture medium following piroxicam treatment. At the same time, internalization of u-PA/u-PAR complexes increased after incubation of chondrocytes with piroxicam or PAI-1 rich SF. CONCLUSION. Our results indicate that the drug induces the surface clearance u-PAR by internalization of u-PA/PAI-/u-PAR complexes. Thus piroxicam reduces both the soluble

fibrinolytic activity of human chondrocytes (increase of PAI activity and decrease of released u-PA) and the cell associated u-PA activity (clearance of u-PAR by internalization). The drug dependent changes in the fibrinolytic system suggest that piroxicam may be useful in preventing or limiting perilacunar cartilage damage in OA.

L17 ANSWER 67 OF 109 MEDLINE on STN
95143384. PubMed ID: 7841301. Soluble **urokinase** receptor from fibrosarcoma HT-1080 cells. Lau H K; Kim M. (Division of Hematology, St Michael's Hospital, Toronto, Ontario, Canada.) Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, **(1994 Aug)** 5 (4) 473-8. Journal code: 9102551. ISSN: 0957-5235. Pub. country: ENGLAND: United Kingdom. Language: English.
AB A soluble form of **urokinase**-binding protein has been isolated from the human fibrosarcoma cell line HT-1080 and cell lines derived from it. Conditioned media of these cells were collected after overnight incubation under serum-free conditions, and were concentrated and passed through a column of Sepharose 4B to which **high-molecular-weight urokinase** had been attached. After thorough washing, a polypeptide could be eluted from the column with 1 M acetic acid. This material appeared to be a single band of approximately 60 kDa on SDS polyacrylamide gel. It cross-reacted with commercial antibodies made against **urokinase** receptor, and could be chemically cross-linked to the **amino terminal fragment** of **urokinase**. This material was similar to the **urokinase** receptor that was cleaved from HT-1080 cells by means of phosphatidylinositol-specific phospholipase C.

L17 ANSWER 68 OF 109 MEDLINE on STN
95101635. PubMed ID: 7803405. Backbone dynamics of a two-domain protein: 15N relaxation studies of the **amino-terminal fragment** of **urokinase**-type plasminogen activator. Hansen A P; Petros A M; Meadows R P; Fesik S W. (Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Ilinois 60064.) Biochemistry, **(1994 Dec 27)** 33 (51) 15418-24. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB The **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (u-PA) is a two-domain protein which consists of a kringle and a growth factor domain (GFD). The dynamics of uniformly 15N-labeled **ATF** was examined by measuring the longitudinal (T1) and transverse (T2) 15N relaxation times and heteronuclear NOEs. The data were interpreted in terms of the model-independent spectral density function. The GFD was found to exhibit a high degree of anisotropy, whereas the kringle domain of **ATF** undergoes isotropic reorientation. This difference in anisotropy is best explained by the two domains moving independently such as differently shaped beads on a string. With the exception of the N- and C-terminal regions of the protein, the most flexible region of **ATF** was the seven-residue omega loop (N22-I28) of the GFD which has been implicated in the binding of u-PA to its receptor. The amides of the linker region between the domains displayed high values of the order parameter, indicating restricted motion on the picosecond time scale. This is in contrast to the flexible linker of calmodulin [Barbato et al. (1992) Biochemistry 31, 5269-5278], which displayed low values of S2 and unrestricted motion in the linker region.

L17 ANSWER 69 OF 109 MEDLINE on STN
95080428. PubMed ID: 7988721. Blockage of the **urokinase** receptor on the cell surface: construction and characterization of a hybrid protein consisting of the N-terminal fragment of human **urokinase** and human albumin. Lu H; Yeh P; Guitton J D; Mabilat C; Desanlis F; Maury I; Legrand Y; Soria J; Soria C. (Unite INSERM 353, Hopital St. Louis, Paris, France.) FEBS letters, **(1994 Dec 12)** 356 (1) 56-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB Receptor-bound **urokinase** is likely to be a crucial determinant in both

tumor invasion and angiogenesis. We report here that a yeast-derived genetic conjugate between human serum albumin and the 1-135 N-terminal residues of **urokinase** (u-PA) competitively inhibits the binding of exogenous and endogenous u-PA to its cell-anchored receptor (u-PAR). This hybrid molecule (**ATF**-HSA) also inhibits in vitro pro-**urokinase**-dependent plasminogen activation in the presence of u-PAR bearing cells. These effects are probably responsible for the observed in vitro inhibition of tumor cell invasion in a reconstituted basement membrane extract (Matrigel).

L17 ANSWER 70 OF 109 MEDLINE on STN

95019438. PubMed ID: 7933839. Receptor binding and degradation of **urokinase**-type plasminogen activator by human mesangial cells. Nguyen G; Li X M; Peraldi M N; Zacharias U; Hagege J; Rondeau E; Sraer J D. (INSERM U 64, Hopital Tenon, Paris, France.) Kidney international, **(1994 Jul)** 46 (1) 208-15. Journal code: 0323470. ISSN: 0085-2538. Pub. country: United States. Language: English.

AB The binding of [125I] labeled **urokinase**-type plasminogen activator (u-PA) was studied on human mesangial cells (MC) in culture. The binding of active [125I]u-PA at 37 degrees C reached a plateau after 30 minutes of incubation and remained stable for at least four hours. When the supernatant was analyzed with trichloracetic acid (TCA), TCA soluble radioactive material could be detected after a lag phase of 30 minutes, and then increased linearly for four hours. Analysis by electrophoresis on SDS PAGE and autoradiography of the cell associated radioactivity and of the intracellular content showed that active u-PA and u-PA complexed to plasminogen activator inhibitor type-1 (PAI-1) were bound to the cell surface, but only u-PA/PAI-1 complexes were internalized and degraded. Therefore, the Kd and the number of binding sites were determined by competitive inhibition curves at 4 degrees C using diisopropyl-fluorophosphate (DFP) u-PA. Scatchard plots showed a Kd = 400 +/- 30 pM, and Bmax = 240,000 +/- 25,000 sites/cell. Excess of the **amino terminal fragment** of u-PA (**ATF**) completely blocked the specific binding of [125I]u-PA, confirming that the binding of u-PA was independent of the presence of the active site and/or of the formation of complexes with PAI-1. 3H thymidine incorporation by mesangial cells after stimulation with 100 nM active u-PA showed that u-PA had a moderate but significant mitogenic effect, in contrast to inactive u-PA and **ATF**. However, this mitogenic effect was not accompanied by a proliferative effect.(ABSTRACT TRUNCATED AT 250 WORDS)

L17 ANSWER 71 OF 109 MEDLINE on STN

94326845. PubMed ID: 8050501. Production of second messengers following chemotactic and mitogenic **urokinase**-receptor interaction in human fibroblasts and mouse fibroblasts transfected with human **urokinase** receptor. Anichini E; Fibbi G; Pucci M; Caldini R; Chevanne M; Del Rosso M. (Institute of General Pathology, Florence University, Italy.) Experimental cell research, **(1994 Aug)** 213 (2) 438-48. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We studied **urokinase**-type plasminogen activator (u-PA)-dependent chemotaxis and DNA synthesis in both human fibroblasts and LB6 mouse fibroblasts transfected with human u-PA receptor (u-PAR) gene (LB6 clone 19). Both cell lines have receptors for the **amino-terminal fragment** of u-PA (u-PA-**ATF**). We observed that u-PA and u-PA-**ATF** stimulated chemotactic migration of both LB6 clone 19 cells and human fibroblasts, which could be impaired by down-regulation of protein kinase C (PKC) with phorbol myristate acetate (PMA). While LB6 clone 19 cells were unable to undergo mitosis following exposure to either u-PA or u-PA-**ATF**, human fibroblasts were stimulated to mitosis by exogenous addition of native u-PA, and u-PA-**ATF** was ineffective. The mitogenic activity of u-PA on human fibroblasts could also be impaired by down-regulation of PKC with PMA. We studied second messenger formation following u-PAR stimulation. Neither inositol lipid metabolism nor intracellular Ca2+ content were

affected, while an increase of diacylglycerol (DAG) generation was observed. Such DAG formation was related to de novo synthesis from glucose and was dependent on ligand-receptor interaction. Both u-PA-**ATF** and the native u-PA molecule were able to stimulate DAG formation, u-PA being from three to fourfold more efficient than **ATF**. These data suggest that u-PAR stimulation per se is sufficient to trigger DAG formation. The native molecule confers on the cell an additional stimulus, possibly related with the activation of a u-PA-catalytic site-dependent substrate. Such stimulation allows the cell to reach the DAG threshold level required to trigger DNA synthesis.

L17 ANSWER 72 OF 109 MEDLINE on STN

94318644. PubMed ID: 8043585. Ligand interaction between **urokinase**-type plasminogen activator and its receptor probed with 8-anilino-1-naphthalenesulfonate. Evidence for a hydrophobic binding site exposed only on the intact receptor. Ploug M; Ellis V; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen O, Denmark.) Biochemistry, **(1994 Aug 2)** 33 (30) 8991-7. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The cellular receptor for **urokinase**-type plasminogen activator (uPAR) is a glycolipid-anchored membrane protein thought to play a primary role in the generation of pericellular proteolytic activity, and to be involved in cancer cell invasion and metastasis. This protein is composed of three homologous domains, the NH2-terminal of which is involved in the high-affinity binding (Kd approximately 0.1-1.0 nM) to the epidermal growth factor-like module of **urokinase**-type plasminogen activator (uPA). Here we report that intact uPAR binds the low molecular weight fluorophore 8-anilino-1-naphthalenesulfonate (ANS) to form a 1:1 stoichiometric complex and that the resulting enhancement of the ANS fluorescence probes the functional state of uPAR as judged by several independent criteria. First, the uPAR-mediated increase in ANS fluorescence can be titrated by uPA as well as by its receptor binding derivatives (the **amino-terminal fragment** and the growth factor-like module). Second, an anti-uPAR monoclonal antibody, capable of preventing uPA binding, can also titrate the uPAR-dependent ANS fluorescence whereas other antibodies not interfering with uPA binding are unable to exert this effect. Third, the dissociation profile of uPA-uPAR complexes as a function of increasing concentrations of guanidine hydrochloride closely parallels the loss of the ANS binding site in uPAR. Finally, liberation of the NH2-terminal domain from uPAR by limited chymotrypsin cleavage after Tyr87 leads to a loss of both enhanced ANS fluorescence and high-affinity uPA binding.(ABSTRACT TRUNCATED AT 250 WORDS)

L17 ANSWER 73 OF 109 MEDLINE on STN

94299497. PubMed ID: 8027043. Protease nexin-1-**urokinase** complexes are internalized and degraded through a mechanism that requires both **urokinase** receptor and alpha 2-macroglobulin receptor. Conese M; Olson D; Blasi F. (Department of Biological and Technological Research, San Raffaele Research Institute, Milano, Italy.) Journal of biological chemistry, **(1994 Jul 8)** 269 (27) 17886-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB After binding to its receptor (uPAR), active cell-surface **urokinase** (uPA) is not internalized while the complex formed by uPA with plasminogen activator inhibitor type 1 (PAI-1) is internalized and degraded. Internalization and degradation require binding to uPAR and subsequently an interaction with the alpha 2-macroglobulin receptor (alpha 2-MR). To analyze the generality of this mechanism, we studied the internalization of uPA by recombinant protease nexin-1 (rPN-1), an inhibitor of thrombin, uPA, and plasmin. 125I-uPA.rPN-1 complexes bound specifically to uPAR; internalization occurred efficiently, and its time course was essentially the same as for uPA.PAI-1. Internalization required binding to uPAR since it could be blocked by the anti-uPAR monoclonal antibodies, by the uPAR antagonist **amino-terminal fragment** of uPA, and by the removal of

uPAR by the treatment of cells with phosphatidylinositol-specific phospholipase C. As for uPA.PAI-1, the internalization of uPA.rPN-1 also required alpha 2-MR, since it could be inhibited by the 39-kDa alpha 2-macroglobulin receptor/low density lipoprotein receptor-associated protein, a ligand for the alpha 2-MR. Finally, we show by ligand blot analysis that the uPA.rPN-1 complex, like uPA.PAI-1 but unlike free uPA, bound specifically to both uPAR and alpha 2-MR.

L17 ANSWER 74 OF 109 MEDLINE on STN

94291776. PubMed ID: 8020601. **Urokinase**-type and tissue-type plasminogen activators as growth factors of human fibroblasts. De Petro G; Copeta A; Barlati S. (Department of Biomedical Sciences and Biotechnologies, University of Brescia, Italy.) Experimental cell research, **(1994 Jul)** 213 (1) 286-94. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB In this study we have verified the mitogenic effect of **urokinase**-type (u-PA) and tissue-type plasminogen activators (t-PA) on human normal fibroblasts. We report that both PAs can induce DNA replication and cell division in serum-deprived cultured human skin fibroblasts. The activity of u-PA and t-PA is, respectively, three- and twofold more potent than that exerted by epidermal growth factor (EGF) with an activity slightly lower (50-60%) than that induced by basic fibroblast growth factor (bFGF). The u-PA and t-PA, but not plasmin, induced DNA synthesis, which could be neutralized by anti-u-PA and anti-t-PA antibodies, respectively, but was insensitive to aprotinin treatment. The addition of anti-u-PA-receptor (u-PAR) monoclonal antibodies to the assays selectively suppressed the mitogenic effect exerted by u-PA, but not that of t-PA, and the **amino-terminal fragment** of u-PA, containing the EGF-like domain and the kringle module, did not elicit any mitogenic activity. Anti-bFGF antibodies completely suppressed the mitogenic activity of bFGF, but did not have any effect on that of u-PA and t-PA; the activity of both PAs was inhibited by anti-fibronectin IgG concentrations ineffective on bFGF. These results indicate that PAs may be considered growth factors of human fibroblasts.

L17 ANSWER 75 OF 109 MEDLINE on STN

94243832. PubMed ID: 8187097. Binding of **urokinase** to its receptor promotes migration and invasion of human melanoma cells in vitro. Stahl A; Mueller B M. (Department of Immunology, Scripps Research Institute, La Jolla, California 92037.) Cancer research, **(1994 Jun 1)** 54 (11) 3066-71. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Previously, we reported that **urokinase**-type plasminogen activator (uPA) plays a pivotal role in extracellular matrix dissolution by malignant melanoma cells. Here, we demonstrate that a highly metastatic melanoma cell line (M24met) that secretes uPA expresses high levels of the uPA receptor (uPAR), 2.4 x 10(6) binding sites/cell with a KD of 5.67 x 10(-10) M. The receptor was identified as a 55,000-60,000 kDa cell surface protein. Although M24met cells secrete uPA, they are unable to efficiently utilize this enzyme for invasion, unless it is bound to its receptor. This contention is based on the finding that an antibody against uPAR (monoclonal antibody 3936) inhibited invasion of M24met cells through a reconstituted basement membrane (Matrigel) up to 33%, while a reduction of uPA catalytic activity by its plasminogen activator inhibitor-2 resulted in 46% inhibition of invasion. Furthermore, uPAR is involved in signal transduction events in M24met cells, since both uPA and its **amino-terminal fragment** stimulated the migration of melanoma cells toward Matrigel, resulting in maximal increases of 32 and 72%, respectively. Our results indicate that both uPA and uPAR are involved in melanoma metastasis and that uPAR contributes to at least two important steps in this process, matrix dissolution and migration.

L17 ANSWER 76 OF 109 MEDLINE on STN

94213863. PubMed ID: 8161544. Solution structure of the **amino-terminal fragment** of **urokinase**-type plasminogen activator. Hansen A P; Petros A M; Meadows R P; Nettesheim D G; Mazar A P; Olejniczak E T; Xu R X; Pederson T M; Henkin J; Fesik S W. (Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois 60064.) Biochemistry, **(1994 Apr 26)** 33 (16) 4847-64. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator is a two domain protein which consists of a growth factor and a kringle domain. The 1H, 13C, and 15N chemical shifts of this protein have been assigned using heteronuclear two- and three-dimensional NMR experiments on selective and uniformly 15N- and 15N/13C-labeled protein isolated from mammalian cells that overexpress the protein. The chemical shift assignments were used to interpret the NOE data which resulted in a total of 1299 NOE restraints. The NOE restraints were used along with 27 phi angle restraints and 21 hydrogen-bonding restraints to produce 15 low energy structures. The individual domains in the structures are highly converged, but the two domains are structurally independent. The root mean square deviations (rmsd) between residues 11-46 in the growth factor domain and the mean atomic coordinates were 0.99 +/- 0.2 for backbone heavy atoms and 1.65 +/- 0.2 for all non-hydrogen atoms. For residues 55-130 in the kringle domain, the rmsd was 0.84 +/- 0.2 for backbone heavy atoms and 1.42 +/- 0.2 for all non-hydrogen atoms. The overall structures of the individual domains are very similar to the structures of homologous proteins. However, important structural differences between the growth factor and other homologous proteins were observed in the region which has been implicated in binding the **urokinase** receptor which may explain, in part, why other growth factors show no appreciable affinity for the **urokinase** receptor.

L17 ANSWER 77 OF 109 MEDLINE on STN

94171881. PubMed ID: 8126064. Requirement for receptor-bound **urokinase** in plasmin-dependent cellular conversion of latent TGF-beta to TGF-beta. Odekon L E; Blasi F; Rifkin D B. (Department of Cell Biology, New York University Medical Center, New York.) Journal of cellular physiology, **(1994 Mar)** 158 (3) 398-407. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB The role of receptor-bound **urokinase**-type plasminogen activator (uPA) in cellular activation of latent transforming growth factor-beta (LTGF-beta) was investigated in a model system of mouse LB6 cells transfected with either a human uPA receptor cDNA (LhuPAR+), a human prouPA cDNA (LhuPA), or a control neomycin-resistance cDNA (Lneo). When LhuPAR+ cells were co-cultured with LhuPA cells, the plasmin-dependent fibrinolytic activity generated was more than that observed in either homotypic cultures with fivefold greater number of LhuPA cells or co-cultures containing LhuPA and Lneo cells instead of the LhuPAR+ cells. The preferential activation of TGF-beta by co-cultures with the greatest plasmin-generation potential, LhuPAR+ and LhuPA cells, was confirmed by three independent bioassays. In the first assay, a 48% decrease in PA activity, a measure of active TGF-beta production, was observed with BAE cells treated with conditioned medium (CM) from co-cultures of LhuPA and LhuPAR+ cells. Inclusion of neutralizing antibodies to TGF-beta abrogated the inhibitory effect of CM on PA activity demonstrating that the inhibitory molecule was TGF-beta. Addition of the **amino terminal fragment** of uPA (**ATF**) or omission of plasminogen from co-cultures blocked both the fibrinolytic activity and the generation of TGF-beta activity in the CM. In the second assay, CM from co-cultures of LhuPA and LhuPAR+ cells inhibited the migration of BAE cells in a wound assay. Controls with anti-TGF-beta IgG indicated that the inhibition was due to TGF-beta. In the third assay, proliferation of mink lung epithelial cells was inhibited by CM generated by co-cultures of LhuPA and LhuPAR+ cells as compared to CM from the same cells cultured in the absence of plasminogen or to CM from a co-culture of LhuPA with LhuPAR- cells. Excess mannose-6-phosphate (M6P) blocked the generation of

TGF-beta as assayed by both the BAE migration and PA assays, presumably because it interfered with cell-surface localization of LTGF-beta. Additionally, small numbers of LhuPA and LhuPAR+ cells co-cultured with BAE cells inhibited the BAE cell PA activity via the paracrine action of TGF-beta. These results support the conclusion that plasmin-dependent activation LTGF-beta by LB6 cells is promoted by the surface localization of uPA by its receptor.

L17 ANSWER 78 OF 109 MEDLINE on STN

94043730. PubMed ID: 8227331. A ligand-free, soluble **urokinase** receptor is present in the ascitic fluid from patients with ovarian cancer. Pedersen N; Schmitt M; Ronne E; Nicoletti M I; Hoyer-Hansen G; Conese M; Giavazzi R; Dano K; Kuhn W; Janicke F; +. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of clinical investigation, **(1993 Nov)** 92 (5) 2160-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB We have identified a soluble form of the **human urokinase plasminogen activator** (uPA) receptor (uPAR) in the ascitic fluids from patients with ovarian cancer. After purification of uPAR from the ascitic fluids by ligand-affinity chromatography (pro-uPA Sepharose), the uPAR was initially identified by cross-linking to a radiolabeled **amino-terminal fragment** of human uPA. The uPAR purified from the ascitic fluid has no bound ligand (uPA), as similar amounts can be purified by ligand-affinity chromatography as by immuno-affinity chromatography. uPAR from ascitic fluids partitions in the water phase after a temperature-dependent phase separation of a detergent extract. It therefore lacks at least the lipid moiety of the glycophospholipid anchor present in cellular-bound uPARs. It is highly glycosylated and the deglycosylated form has the same electrophoretic mobility as previously characterized cellular uPAR from other sources. The immunoreactivity of the purified uPAR from the ascitic fluid is indistinguishable from that of characterized uPAR, demonstrated by Western blotting with three different anti-uPAR monoclonal antibodies. The uPAR was found in 11 of 11 ascitic fluids from patients with ovarian cancer and in elevated amounts in the plasma from 2 of 3 patients. The concentration of soluble uPAR in the ascitic fluid was estimated to range between 1 and 10 ng/ml. Human soluble uPAR, derived from the tumor cells, was also found in the ascitic fluid and serum from nude mice xenografted intraperitoneally with three different human ovarian carcinomas.

L17 ANSWER 79 OF 109 MEDLINE on STN

94012773. PubMed ID: 7691818. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates cellular uptake of pro-**urokinase**. Kounnas M Z; Henkin J; Argraves W S; Strickland D K. (Biochemistry Laboratory, American Red Cross, Rockville, Maryland 20855.) Journal of biological chemistry, **(1993 Oct 15)** 268 (29) 21862-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) is a large cell surface receptor consisting of a 515-kDa heavy chain and an 85-kDa light chain proteolytically derived from a 600-kDa precursor. Previous work has shown that LRP is responsible for mediating the internalization of urinary-type plasminogen activator (uPA) complexed to plasminogen activator inhibitor type I (PAI-1) (Nykjaer et al., 1992; Herz et al., 1992). The current study indicates that pro-**urokinase** (pro-uPA) and two chain **urokinase** (tc-uPA) bind directly to purified LRP, and that LRP mediates their internalization and degradation in Hep G2 cells. In vitro binding assays demonstrated that pro-uPA and tc-uPA bind to purified LRP with affinities (Kd = 45 and 60 nM, respectively) that are approximately 15 to 20-fold weaker than the affinity of uPA.PAI-1 complex for LRP (Kd = 3 nM). Competitive binding experiments revealed that pro-uPA and tc-uPA completely inhibit binding of uPA.PAI-1 complexes to purified LRP. The binding of 125I-pro-uPA to LRP is blocked by the 39-kDa receptor-associated protein, but not by an

amino-terminal fragment of uPA, which is known to block binding of uPA to the **urokinase** receptor. 125I-Pro-uPA can be internalized and degraded by Hep G2 cells independent of PAI-1. Both the internalization and degradation are completely blocked by receptor-associated protein or affinity-purified LRP antibodies, indicating that LRP is mediating this process. These processes are also blocked by the **amino-terminal fragment**, which suggests that the favored pathway for uPA metabolism is initial binding to the **urokinase** receptor, followed by ligand transfer to LRP, then internalization leading to degradation.

L17 ANSWER 80 OF 109 MEDLINE on STN

93272971. PubMed ID: 8388810. Processing of complex between **urokinase** and its type-2 inhibitor on the cell surface. A possible regulatory mechanism of **urokinase** activity. Ragno P; Montuori N; Vassalli J D; Rossi G. (Centro di Endocrinologia ed Oncologia Sperimentale (CEOS), Consiglio Nazionale delle Ricerche, Naples, Italy.) FEBS letters, **(1993 Jun 1)** 323 (3) 279-84. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Complexes between the **urokinase**-type plasminogen activator (uPA) and its type-2 inhibitor (PAI-2) are bound by a cell-surface receptor for uPA and rapidly cleaved into two fragments of 70 and 22 kDa. The 70-kDa fragment contains the active site of uPA and PAI-2, while the 22-kDa species was identified as the **amino terminal fragment** of uPA, that binds specifically to the receptor. When the experiment is performed at 4 degrees C, both fragments remain bound to the cell surface and can be eluted by acid treatment. We therefore postulate that after the binding of the uPA-PAI-2 complex, a new binding site for the 70-kDa species becomes available. This additional binding favours the cleavage of the complex into the 70-and 22-kDa fragments; the 70-kDa species is endocytosed or released, while the 22-kDa fragment remains on the cell surface to prevent the binding of intact uPA.

L17 ANSWER 81 OF 109 MEDLINE on STN

93261817. PubMed ID: 8388098. Purification and cDNA cloning of a transcription factor which functionally cooperates within a cAMP regulatory unit in the porcine uPA gene. Menoud P A; Matthies R; Hofsteenge J; Nagamine Y. (Friedrich Miescher-Institut, Basel, Switzerland.) Nucleic acids research, **(1993 Apr 25)** 21 (8) 1845-52. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB One of cAMP-regulatory sites in the porcine **urokinase**-type plasminogen activator (uPA) gene resides 3.4 kb upstream of the transcription initiation site and is composed of three protein binding domains, FPA, FPB and FPC. Whereas FPA and FPB contain a CRE-like sequence, the FPC sequence is not related to any known protein recognition sequences, yet all three domains are required to mediate cAMP action on a heterologous promoter. To study the functional cooperation among these three domains we purified and cloned a FPC-binding protein (FPCB) from porcine kidney derived LLC-PK1 cells. Sequence comparisons showed that FPCB is homologous to mouse LFB3 and rat vHNF1. LFB3/vHNF1 is related to a liver specific transcription factor HNF1, it recognizes the same sequence as HNF1 and is highly expressed in kidney cells. FPCB and HNF1 recognition sequences are dissimilar, nevertheless both sequences are recognized by in vitro-translated LFB3 and FPCB, indicating that binding to the two different sequences is an intrinsic character of FPCB/LFB3/vHNF1. In HeLa cells, this cAMP-responsive site was inactive whether FPCB was overexpressed or not, suggesting a requirement for an additional cell-specific factor. These results may suggest a mechanism by which hormonal control is integrated into cell-specific gene regulation.

L17 ANSWER 82 OF 109 MEDLINE on STN

93176695. PubMed ID: 8382511. Saturation of tumour cell surface receptors for **urokinase**-type plasminogen activator by **amino-terminal**

fragment and subsequent effect on reconstituted basement membranes invasion. Kobayashi H; Ohi H; Shinohara H; Sugimura M; Fujii T; Terao T; Schmitt M; Goretzki L; Chucholowski N; Janicke F; +. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Shizuoka, Japan.) British journal of cancer, **(1993 Mar)** 67 (3) 537-44. Journal code: 0370635. ISSN: 0007-0920. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Single-chain **urokinase**-type plasminogen activator (pro-uPA) is bound to a specific surface receptor on ovarian cancer HOC-I cells that is incompletely saturated. Saturation of uncovered receptors by uPA polypeptides with intact **amino-terminal fragment** (**ATF**) derived from pro-uPA by limited proteolysis (human leucocyte elastase [HLE] or V8 protease) has been studied. HOC-I cells preferentially invaded reconstituted basement membranes in a time- and plasminogen-dependent manner. This process was inhibitable by preincubation with uPA polypeptides in the medium at levels which suggested that complete saturation of cell surface uPA receptors occurred. This result indicates that occupation of uPA receptors by enzymatically inactive uPA fragments or prevention of rebinding of pro-uPA synthesised by tumour cells to the receptors specifically reduces the invasion of the tumour cells through basement membranes in vitro.

L17 ANSWER 83 OF 109 MEDLINE on STN

93151813. PubMed ID: 8381273. **Urokinase-urokinase** receptor interaction: non-mitogenic signal transduction in human epidermal cells. Del Rosso M; Anichini E; Pedersen N; Blasi F; Fibbi G; Pucci M; Ruggiero M. (Institute of General Pathology, Firenze, Italy.) Biochemical and biophysical research communications, **(1993 Jan 29)** 190 (2) 347-52. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB We studied non-mitogenic signal transduction in a human cell line of epidermal origin which is induced to chemotaxis following stimulation with human **urokinase**-type plasminogen activator (u-PA) or with the **amino-terminal fragment** (**ATF**) of u-PA A chain, which specifically interacts with the cellular receptor. U-PA and **ATF** stimulated the formation of diacylglycerol (DAG) independently of inositol lipid and phosphatidylcholine turnover, but concomitantly with de novo synthesis from glucose, thus resembling the DAG neosynthesis activated by insulin. DAG was measured in normal epidermal cells and in cells transfected with the human u-PA receptor (u-PAR) gene and stimulated with u-PA or **ATF**. Transfected clones showed an increase of cell motility under an **ATF** gradient in vitro as well as an increase of DAG production. These findings identify a novel mechanism of second messenger formation that conveys chemotactic signals upon stimulation of the u-PAR.

L17 ANSWER 84 OF 109 MEDLINE on STN

93093104. PubMed ID: 1333982. Selective localization of receptors for **urokinase amino-terminal fragment** at substratum contact sites of an in vitro-established line of human epidermal cells. Del Rosso M; Pedersen N; Fibbi G; Pucci M; Dini G; Anichini E; Blasi F. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Experimental cell research, **(1992 Dec)** 203 (2) 427-34. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We have shown the presence of surface receptors for the **amino-terminal fragment** (**ATF**) of human **urokinase**-type plasminogen activator (u-PA) on an in vitro-established cell line of human epidermal origin by both radio-binding assays with human 125I-u-PA-**ATF** and transmission electron microscopy of a gold-u-PA complex. On the basis of cross-linking experiments with 125I-u-PA-**ATF** and subsequent autoradiography of the gels we have observed that such receptors are not spontaneously released into the culture medium. The treatment with phosphatidylinositol-specific phospholipase C induces the release of the receptor, which behaves as a glycosyl phosphatidyl inositol(GPI)-anchored protein. Phase-partitioning

experiments on cell lysates have shown that the receptor partitions into the detergent phase. By detaching cell monolayers with the chelating agent EDTA we have prepared the cell-substratum contact sites of these cells, which represent only the 3.5% of the surface membrane of monolayered cells. Such plasma membrane remnants are highly selected since they contain about 43% of total u-PA-**ATF** binding sites. Such binding sites show the same biochemical and morphological characteristics of u-PA-**ATF** receptors observed in the monolayered cells, thus indicating that u-PA is selectively concentrated at the level of cell-substratum contacts. This is likely to enable directional proteolysis for cell migration and invasion.

L17 ANSWER 85 OF 109 MEDLINE on STN

92378989. PubMed ID: 1510944. Heparin binding to the **urokinase** kringle domain. Stephens R W; Bokman A M; Myohanen H T; Reisberg T; Tapiovaara H; Pedersen N; Grondahl-Hansen J; Llinas M; Vaheri A. (Department of Virology, University of Helsinki, Finland.) Biochemistry, **(1992 Aug 25)** 31 (33) 7572-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The binding of **urokinase** to immobilized heparin and dextran sulfate was studied using activity assays of the bound **urokinase**. The markedly higher binding observed with high M(r) **urokinase** compared to low M(r) **urokinase** indicated a role for the **amino-terminal fragment** (**ATF**). This was confirmed by the use of inactive truncated **urokinase** and monoclonal antibodies specific for the **ATF** in competition assays of **urokinase** binding. Antibody competition assays suggested a site in the kringle domain, and a synthetic decapeptide Arg-52-Trp-62 from the kringle sequence (kringle numbering convention) was competitive in assays of **urokinase** binding to dextran sulfate and heparin. Heparin binding to the **urokinase** kringle was unambiguously demonstrated via 1H NMR spectroscopy at 500 MHz. Effective equilibrium association constants (K(a)*) were determined for the interaction of isolated kringle fragment and low M(r) heparin at pH 7.2. The binding was strong in salt-free 2H2O (K(a)* approximately 57 mM-1) and remained significant in 0.15 M NaCl (K(a)* approximately 12 mM-1), supporting a potential physiological role for the interaction. This is the first demonstration of a function for the kringle domain of **urokinase**, and it suggests that while the classical kringle structure has specificity for lysine binding, there may also exist a class of kringles with affinity for polyanion binding.

L17 ANSWER 86 OF 109 MEDLINE on STN

92362631. PubMed ID: 1499567. **Urokinase** binding to laminin-nidogen. Structural requirements and interactions with heparin. Stephens R W; Aumailley M; Timpl R; Reisberg T; Tapiovaara H; Myohanen H; Murphy-Ullrich J; Vaheri A. (Department of Virology, University of Helsinki, Finland.) European journal of biochemistry / FEBS, **(1992 Aug 1)** 207 (3) 937-42. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Recently we have shown that heparin and related sulfated polyanions are low-affinity ligands of the kringle domain in the amino-terminal region (**ATF**) of human **urokinase** (u-PA), and proposed that this may facilitate loading of u-PA onto its receptor at the focal contacts between adherent cells and their matrix. We have now tested other components of the cell matrix (fibronectin, vitronectin, thrombospondin and laminin-nidogen) for u-PA binding, and found that laminin-nidogen is also a ligand of the u-PA **ATF**. Direct binding assays and competition binding assays with defined fragments of laminin-nidogen showed that there are u-PA binding sites in fragment E4 of laminin as well as in nidogen. The long-arm terminal domain of laminin (fragment E3), which contains a heparin-binding site, competed for binding of u-PA to immobilised heparin. However nidogen, which does not bind to heparin, also inhibited binding of u-PA to heparin, and this effect was also observed with recombinant nidogen and with a fragment of nidogen lacking the carboxy-terminal domain. Direct binding

assays confirmed that u-PA binds to nidogen through a site in the u-PA **ATF**. We conclude that u-PA binds to laminin-nidogen by interactions involving the **ATF** region of u-PA, the E4 domain of laminin and the rod or amino-terminal regions of nidogen. Since nidogen is suggested to be an important bridging molecule in the maintenance of the supramolecular organization in basement membranes, the presence of a binding site for u-PA in nidogen indicates a role for plasminogen activation in basement membrane remodelling.

L17 ANSWER 87 OF 109 MEDLINE on STN

92340481. PubMed ID: 1378833. Purified alpha 2-macroglobulin receptor/LDL receptor-related protein binds **urokinase**.plasminogen activator inhibitor type-1 complex. Evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of **urokinase** receptor-bound complexes. Nykjaer A; Petersen C M; Moller B; Jensen P H; Moestrup S K; Holtet T L; Etzerodt M; Thogersen H C; Munch M; Andreasen P A; +. (Institute of Medical Biochemistry, University of Aarhus, Denmark.) Journal of biological chemistry, **(1992 Jul 25)** 267 (21) 14543-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Complexes between 125I-labeled **urokinase**-type plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) bound to purified alpha 2-macroglobulin (alpha 2M) receptor (alpha 2MR)/low density lipoprotein receptor-related protein (LRP). No binding was observed when using uPA. The magnitude of uPA.PAI-1 binding was comparable with that of the alpha 2MR-associated protein (alpha 2MRAP). Binding of uPA.PAI-1 was blocked by natural and recombinant alpha 2MRAP, and about 80% inhibited by complexes between tissue-type plasminogen activator (tPA) and PAI-1, and by a monoclonal anti-PAI-1 antibody. In human monocytes, uPA.PAI-1, like uPA and its **amino-terminal fragment**, bound to the **urokinase** receptor (uPAR). Degradation of uPAR-bound 125I-uPA.PAI-1 was 3-4-fold enhanced as compared with uncomplexed uPAR-bound uPA. The inhibitor-enhanced uPA degradation was blocked by r alpha 2MRAP and inhibited by polyclonal anti-alpha 2MR/LRP antibodies. This is taken as evidence for mediation of internalization and degradation of uPAR-bound uPA.PAI-1 by alpha 2MR/LRP.

L17 ANSWER 88 OF 109 MEDLINE on STN

92332522. PubMed ID: 1321137. Structural requirements for the growth factor activity of the amino-terminal domain of **urokinase**. Rabbani S A; Mazar A P; Bernier S M; Haq M; Bolivar I; Henkin J; Goltzman D. (Department of Medicine, Royal Victoria Hospital, McGill University, Montreal, Canada.) Journal of biological chemistry, **(1992 Jul 15)** 267 (20) 14151-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **High molecular weight urokinase-type plasminogen activator** (uPA) in which proteolytic activity was inactivated (diisopropyl fluorophosphate (DFP)-uPA), its **amino-terminal fragment** (**ATF**, amino acids (aa) 1-143), and fucosylated and defucosylated growth factor domains (GFD, aa 4-43) were tested for growth-promoting effects and binding in human SaOS-2 osteosarcoma cells and U-937 lymphoma cells. DFP-uPA, **ATF**, and both the fucosylated and defucosylated GFD were capable of competing with 125I-**ATF** for binding to both SaOS-2 and U-937 cells. DFP-uPA, **ATF**, and fucosylated GFD were also mitogenic in SaOS-2 cells and increased cell numbers. However, defucosylated GFD was nonmitogenic in SaOS-2 cells and did not stimulate cell proliferation, even though it bound to these cells in a manner equivalent to the fucosylated GFD. A nonglycosylated high molecular weight uPA expressed and purified from Escherichia coli inhibited 125I-**ATF** binding to SaOS-2 cells but was also nonmitogenic. No mitogenic activity was observed in U-937 cells treated with the uPA forms capable of eliciting a mitogenic response in SaOS-2 cells. Proteolytically prepared kringle domain (aa 47-135) and low molecular weight uPA (aa 144-411) did not compete for 125I-**ATF** binding and did not elicit any mitogenic response in either of

the cell lines tested. In addition, tissue plasminogen activator (tPA), which has been shown to be homologous to uPA in its growth factor domain and is also fucosylated, did not inhibit 125I-**ATF** binding nor elicit any mitogenic response. These results demonstrate that the GFD, implicated in binding to the uPA receptor, is also responsible for growth factor like activity in SaOS-2 cells and that the fucosylation at Thr18 within this domain may serve as a molecular trigger in eliciting this response.

L17 ANSWER 89 OF 109 MEDLINE on STN

92250509. PubMed ID: 1315748. Internalization of the **urokinase**-plasminogen activator inhibitor type-1 complex is mediated by the **urokinase** receptor. Olson D; Pollanen J; Hoyer-Hansen G; Ronne E; Sakaguchi K; Wun T C; Appella E; Dano K; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of biological chemistry, **(1992 May 5)** 267 (13) 9129-33. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The role of the **urokinase** receptor (uPAR) in the internalization of the **urokinase**-plasminogen activator inhibitor type-1 (uPA.PAI-1) complex has been investigated. First, exploiting the species specificity of uPA binding, we show that mouse LB6 cells (that express a mouse uPAR) were unable to bind or degrade the human uPA.PAI-1 complex. On the other hand, LB6 clone 19 cells, which express a transfected human uPAR, degraded uPA.PAI-1 complexes with kinetics identical to the human monocytic U937 cells. We also show by immunofluorescence experiments with anti-uPA antibodies that in LB6 clone 19 cells, the uPA.PAI-1 complex is indeed internalized. While at 4 degrees C uPA fluorescence was visible at the cell surface, shift of the temperature to 37 degrees C caused a displacement of the immunoreactivity to the cytoplasmic compartment, with a pattern indicating lysosomal localization. If uPA.PAI-1 internalization/degradation is mediated by uPAR, inhibition of uPA.PAI-1 binding to uPAR should block degradation. Three different treatments, competition with the agonist **amino-terminal fragment** of uPA, treatment with a monoclonal antibody directed toward the binding domain of uPAR or release of uPAR from the cell surface with phosphatidylinositol-specific phospholipase C completely prevented uPA.PAI-1 degradation. The possibility that a serpin-enzyme complex receptor might be primarily or secondarily involved in the internalization process was excluded since a serpin-enzyme complex peptide failed to inhibit uPA.PAI-1 binding and degradation. Similarly, complexes of PAI-1 with low molecular mass uPA (33 kDa uPA), which lacks the uPAR binding domain, were neither bound nor degraded. Finally we also show that treatment of cells with uPA.PAI-1 complex caused a specific but partial down-regulation of uPAR. A similar result was obtained when PAI-1 was allowed to complex to uPA that had been previously bound to the receptor. The possibility therefore exists that the entire complex uPA.PAI-1-uPAR is internalized. All these data allow us to conclude that internalization of the uPA.PAI-1 complex is mediated by uPAR.

L17 ANSWER 90 OF 109 MEDLINE on STN

92210602. PubMed ID: 1313432. Demonstration of a specific clearance receptor for tissue-type plasminogen activator on rat Novikoff hepatoma cells. Nguyen G; Self S J; Camani C; Kruithof E K. (Division of Hematology, Centre Hospitalia Universitaire Vaudois, Lausanne, Switzerland.) Journal of biological chemistry, **(1992 Mar 25)** 267 (9) 6249-56. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The binding, internalization, and degradation of tissue-type plasminogen activator (t-PA) were studied in a rat hepatoma (Novikoff) cell line. Binding of t-PA to specific saturable high affinity binding sites (Kd = 12 nM, 54,000 sites/cell) was followed by internalization and degradation and did not require a functional active site. The catabolism of t-PA was not inhibited by an excess of **urokinase**-type plasminogen activator (u-PA), and t-PA bound to Novikoff membranes was not complexed to PAI-1,

suggesting a mechanism independent of PAI-1. Additionally, a mannose receptor is not involved since t-PA binding was not influenced by an excess of mannose, galactose, ovalbumin, or EDTA. Furthermore, the degradation of t-PA was not influenced by 10 mM 6-aminohexanoic acid, a lysine analogue. The t-PA receptor binds to and can be eluted from wheat germ agglutinin-Sepharose. Cross-linking of t-PA with partially purified receptor and ligand blot analysis, suggest that t-PA binds to two proteins, a principal one of 55 kDa and a minor one of 43 kDa. Novikoff cells are able also to bind (Kd = 1.4 nM, 25,000 sites/cell) and degrade u-PA. The binding was inhibited by pro-u-PA and the **amino-terminal fragment** of u-PA, but not by an excess of t-PA. The u-PA receptor, but not the t-PA receptor, was removed by treatment with phosphatidylinositol-specific phospholipase C. Our results show that the clearance receptor for t-PA on Novikoff cells is different from the mannose receptor and the PAI-1-dependent receptor described in other cells. The rat hepatoma cells are thus a good model to study the PAI-1 independent hepatocyte-specific clearance of t-PA.

L17 ANSWER 91 OF 109 MEDLINE on STN

92198395. PubMed ID: 1801751. Biological and clinical relevance of the **urokinase**-type plasminogen activator (uPA) in breast cancer. Schmitt M; Goretzki L; Janicke F; Calvete J; Eulitz M; Kobayashi H; Chucholowski N; Graeff H. (Frauenklinik, Technischen Universitat Munchen, Klinikum rechts der Isar, FRG.) Biomedica biochimica acta, **(1991)** 50 (4-6) 731-41. Journal code: 8304435. ISSN: 0232-766X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Tumor cell invasion and metastasis is a multifactorial process, which at each step may require the action of proteolytic enzymes such as collagenases, cathepsins, plasmin, or plasminogen activators. An enzymatically inactive proenzyme form of the **urokinase**-type plasminogen activator (pro-uPA) is secreted by tumor cells which may be converted to an enzymatically active two-chain uPA-molecule (**HMW-uPA**) by plasmin-like enzymes. Action of proteases on pro-uPA may generate the enzymatically active or inactive high-molecular-weight form of uPA (**HMW-uPA**). Some proteases (plasmin, cathepsin B and L, kallikrein, trypsin or thermolysin) activate pro-uPA by cleaving the peptide bond Lys158 and IIe159. Other proteases (elastase, thrombin) cleave pro-uPA at different positions to yield enzymatically inactive **HMW-uPA**. **HMW-uPA** may be split into the enzymatically active LMW-uPA and the enzymatically inactive **ATF** (**amino terminal fragment**). **ATF** may be cleaved between peptide sequence 20 and 40 within the receptor binding domain of uPA (GFD). Such impaired **ATF** does not bind to uPA-receptors. Action of the bacterial endoproteinase Asp-N from Pseudomonas fragi mutant on pro-uPA or **HMW-uPA**, however, generates intact **ATF** which efficiently competes for binding of **HMW-uPA** or pro-uPA to receptors on tumor cells. High uPA-antigen content (pro-uPA, **HMW-uPA**, or LMW-uPA) in breast cancer tissue (not in plasma) indicates an elevated risk for the patient of recurrences and shorter overall survival. Thus pro-uPA/uPA-antigen content in breast cancer tissue serves as an independent prognostic parameter for the outcome of the disease. Cathepsin D is also an independent prognostic factor for recurrences and overall survival. High content of cathepsin D in breast cancer tumors is, however, not correlated with elevated levels of pro-uPA/uPA indicating that synthesis and release of cathepsin D and pro-uPA/uPA are independent events.

L17 ANSWER 92 OF 109 MEDLINE on STN

92129448. PubMed ID: 1734031. **Urokinase**-type plasminogen activator mediates basic fibroblast growth factor-induced bovine endothelial cell migration independent of its proteolytic activity. Odekon L E; Sato Y; Rifkin D B. (Department of Cell Biology, New York University Medical Center, New York.) Journal of cellular physiology, **(1992 Feb)** 150 (2) 258-63. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United

States. Language: English.

AB The dependence of **urokinase**-type plasminogen activator (uPA) induction on endogenous basic fibroblast growth factor (bFGF) activity during endothelial cell migration was investigated utilizing a combination of wounded endothelial cell monolayers and substrate overlay techniques. Purified polyclonal rabbit immunoglobulin G (IgG) against bFGF blocked the appearance of uPA-dependent lytic activity normally observed at the edge of a wounded bovine aortic endothelial (BAE) cell monolayer. Additionally, the migration of cells into the denuded area was inhibited 30-50% by antibodies either to bFGF or to bovine uPA. Incubation of wounded monolayers with either purified bovine uPA or agents able to induce PA activity, such as phorbol myristate acetate (PMA), vanadate, or bFGF, resulted in enhanced migration of cells (28-50%). Anti-bovine uPA IgG blocked a significant fraction (25%) of BAE cell migration induced by exposure to exogenous bFGF. The role of uPA in migration of wounded BAE cells was not dependent on plasmin generation. Furthermore, the **amino terminal fragment** (**ATF**) of human recombinant (hr) uPA, which is enzymatically inactive, stimulated endothelial cell movement in the presence of anti-bFGF IgG. These results suggest that BAE cell migration from the edge of a wounded monolayer is dependent upon local increases of uPA mediated by endogenous bFGF. Moreover, the data support the conclusion that migration is stimulated via a signalling mechanism dependent upon occupancy of the uPA receptor but independent of uPA-mediated proteolysis.

L17 ANSWER 93 OF 109 MEDLINE on STN

91224949. PubMed ID: 1851152. A soluble, ligand binding mutant of the **human urokinase plasminogen activator** receptor. Masucci M T; Pedersen N; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of biological chemistry, **(1991 May 15)** 266 (14) 8655-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A truncated version of the **human urokinase plasminogen activator** receptor has been obtained by in vitro mutagenesis by insertion of a premature nonsense codon in the **urokinase** plasminogen activator receptor cDNA. This results in a protein truncated immediately upstream of the region which appears to be required for membrane attachment of the receptor via a glycolipid anchor. The modified receptor cDNA inserted into an expression vector has been transfected into mouse LB6 cells. Transfectants produce a **urokinase** plasminogen activator (u-PA)-binding protein that is secreted into the medium. It can be cross-linked to iodinated **ATF** (**amino-terminal fragment** of u-PA) and can also inhibit binding of iodinated **ATF** to mouse LB6 cells that express the wild type human receptor. The soluble u-PA receptor will be used in a variety of experiments aimed at identifying the role and mechanism of u-PA in physiological and pathological invasive processes, as well as in therapeutical attempts to block or decrease cancer cell invasion and in general u-PA-mediated tissue destruction.

L17 ANSWER 94 OF 109 MEDLINE on STN

91154379. PubMed ID: 1847936. An autocrine role for **urokinase** in phorbol ester-mediated differentiation of myeloid cell lines. Nusrat A R; Chapman H A Jr. (Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115.) Journal of clinical investigation, **(1991 Mar)** 87 (3) 1091-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB The human myeloid cell line HL60 secretes **urokinase**-type plasminogen activator (uPA) and expresses its receptor. When stimulated with phorbol myristate acetate (PMA), both secretion of uPA and the expression of its receptor are up-regulated, and these cells differentiate to an adherent phenotype. This adhesive response is markedly reduced in the presence of uPA antibodies. The PMA response is restored by the addition of native uPA, an **amino-terminal fragment** of uPA (residues 1-143) devoid of

proteolytic activity, or a synthetic peptide (residues 12-32) from the uPA growth factor domain known to mediate receptor binding. In contrast, the addition of catalytically active low molecular weight uPA, which is missing the growth factor domain, or a peptide from the catalytic domain (residues 247-266) is ineffective. The influence of uPA antibodies on a second marker of macrophage differentiation, cysteine proteinase activity, was also examined. Cysteine proteinase activity of HL60 cells is increased in PMA-treated cells after 24 h but it fails to increase in the presence of anti-uPA. This increase in cathepsin B-like activity is also restored by exogenous uPA. These experiments indicate that an autocrine interaction of the growth factor domain of uPA with its receptor mediates an essential step in PMA-mediated myeloid cell differentiation.

L17 ANSWER 95 OF 109 MEDLINE on STN

91107700. PubMed ID: 1846368. Cellular receptor for **urokinase** plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. Ploug M; Ronne E; Behrendt N; Jensen A L; Blasi F; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of biological chemistry, **(1991 Jan 25)** 266 (3) 1926-33. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The cellular receptor for human **urokinase**-type plasminogen activator (u-PAR) is shown by several independent criteria to be a true member of a family of integral membrane proteins, anchored to the plasma membrane exclusively by a COOH-terminal glycosyl-phosphatidylinositol moiety. 1) Amino acid analysis of u-PAR after micropurification by affinity chromatography and N-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed the presence of 2-3 mol of ethanolamine/mol protein. 2) Membrane-bound u-PAR is efficiently released from the surface of human U937 cells by trace amounts of purified bacterial phosphatidylinositol-specific phospholipase C. This soluble form of u-PAR retains the binding specificity toward both u-PA and its **amino-terminal fragment** holding the receptor-binding domain. 3) Treatment of purified u-PAR with phosphatidylinositol-specific phospholipase C or mild alkali completely alters the hydrophobic properties of the receptor as judged by temperature-induced detergent-phase separation and charge-shift electrophoresis. 4) Biosynthetic labeling of u-PAR was obtained with [3H]ethanolamine and myo-[3H]inositol. 5) Finally, comparison of amino acid compositions derived from cDNA sequence and amino acid analysis shows that a polypeptide of medium hydrophobicity is excised from the COOH terminus of the nascent u-PAR. A similar proteolytic processing has been reported for other proteins that are linked to the plasma membrane by a glycosyl-phosphatidylinositol membrane anchor.

L17 ANSWER 96 OF 109 MEDLINE on STN

91097529. PubMed ID: 2125213. An **amino-terminal fragment** of **urokinase** isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. Rabbani S A; Desjardins J; Bell A W; Banville D; Mazar A; Henkin J; Goltzman D. (Department of Physiology, McGill University, Montreal, Quebec, Canada.) Biochemical and biophysical research communications, **(1990 Dec 31)** 173 (3) 1058-64. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB A peptide mitogen for cultured osteoblast-like cells was purified from serum-free conditioned culture medium of a human prostatic cancer cell line, PC-3. Based on amino acid sequencing and estimated molecular weight, this peptide was identified as an NH2-terminal fragment of **urokinase**-type plasminogen activator (uPA). Recombinant high molecular weight (**HMW**) **uPA** and the NH2-terminal growth factor domain (GFD) of uPA, but not low molecular weight (LMW) uPA (lacking the NH2-terminal region) stimulated [3H] thymidine incorporation and proliferation in osteoblast-like cells, and specific, competitive binding sites for HMW, but not LMW, uPA were demonstrable. These studies demonstrate the

production of a mitogenic NH2-terminal fragment of uPA by a human prostatic cancer cell line which may be of importance in the pathogenesis of osteoblastic metastases.

L17 ANSWER 97 OF 109 MEDLINE on STN

91083011. PubMed ID: 2175557. Alveolar macrophage **urokinase** receptors localize enzyme activity to the cell surface. Chapman H A; Bertozzi P; Sailor L Z; Nusrat A R. (Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.) American journal of physiology, **(1990 Dec)** 259 (6 Pt 1) L432-8. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB Human alveolar macrophages are known to synthesize **urokinase** (uPA) and a specific plasminogen activator inhibitor, PAI-2. In this study we have identified a uPA receptor expressed by these cells and defined the influence of PAI-2 on the interaction of uPA with its receptor. Alveolar macrophages from four normal volunteers were incubated with 55 kDa 125I-labeled uPA (0.24-8 nM) in the presence or absence of excess unlabeled uPA. Specific and saturable binding was demonstrable in all cases. Scatchard plots were linear; regression analysis revealed a mean Kd of 5.25 nM (range 3.2-6.7) and mean Bmax of 30.7 femtomoles/10(5) cells (range 21.5-34.5). The structure of the uPA receptor was defined by electroblotting membrane fractions of macrophages and sequentially exposing filters to uPA and uPA antibodies. Membranes from macrophages demonstrated binding of either uPA or a 15-kDa **amino-terminal fragment** of uPA to a 55- to 60-kDa glycosylated membrane protein. Binding of uPA to filters was blocked by a synthetic oligopeptide containing the known receptor binding region of native uPA. Preincubation of 125I-uPA with PAI-2 dramatically reduced the rate of association of uPA with macrophage uPA receptor. Conversely, receptor-bound uPA activity was less susceptible to inhibition by PAI-2 than soluble uPA activity. These data indicate that normal alveolar macrophages express uPA receptors. The receptor preferentially binds and protects free uPA over complexed enzyme, indicating that one function of the receptor is to allow the cells to express active uPA in an inhibitor-rich environment.

L17 ANSWER 98 OF 109 MEDLINE on STN

90214616. PubMed ID: 2157592. Receptor-mediated internalization and degradation of **urokinase** is caused by its specific inhibitor PAI-1. Cubellis M V; Wun T C; Blasi F. (Biotechnology Center for Molecular Cell Biology, University of Copenhagen, Denmark.) EMBO journal, **(1990 Apr)** 9 (4) 1079-85. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The receptor for **urokinase** plasminogen activator (uPA) has been previously shown not to internalize its ligand, but rather to focalize its activity at the cell surface, allowing a regulated cell surface plasmin dependent proteolysis. The receptor in fact binds the proenzyme pro-uPA and allows its very efficient conversion to the active two chains form. Receptor bound active uPA can also interact with its specific type 1 inhibiror (PAI-1) which is therefore able to inhibit the cell surface plasmin formation. In this paper we show that the uPA-PAI-1 complex bound to the uPA receptor is internalized and degraded. U937 cells were incubated at 4 degrees C with labeled uPA-PAI-1 (and other ligands), the temperature then raised to 37 degrees C and the fate of the ligand followed for 3 h thereafter. The uPA-PAI-1 complex was internalized into the cells (i.e. could not be dissociated by acid treatment) and thereafter degraded (i.e. appeared in the supernatant in a non TCA-precipitable form). Other ligands (free uPA, **ATF** and DFP-treated uPA) were not internalized nor degraded. The degradation of the uPA-PAI-1 complex is preceded by internalization and is inhibited by chloroquine, an inhibitor of lysosomal protein degradation. These data suggest the existence of a cellular cycle of uPA. After synthesis pro-uPA is secreted, bound to the receptor and activated to two chain uPA. On the surface, uPA can activate surface bound plasminogen to produce surface bound plasmin. In the

presence of PAI-1 uPA activity is inhibited and plasmin production interrupted, while the uPA-PAI-1 complex is internalized and degraded.

L17 ANSWER 99 OF 109 MEDLINE on STN

90202929. PubMed ID: 2156852. The human receptor for **urokinase** plasminogen activator. NH2-terminal amino acid sequence and glycosylation variants. Behrendt N; Ronne E; Ploug M; Petri T; Lober D; Nielsen L S; Schleuning W D; Blasi F; Appella E; Dano K. (Finsen Laboratory, Righospitalet, Copenhagen, Denmark.) Journal of biological chemistry, **(1990 Apr 15)** 265 (11) 6453-60. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The receptor for human **urokinase**-type plasminogen activator (u-PA) was purified from phorbol 12-myristate 13-acetate-stimulated U937 cells by temperature-induced phase separation of detergent extracts, followed by affinity chromatography with immobilized diisopropyl fluorophosphate-treated u-PA. The purified protein shows a single 55-60 kDa band after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining. It is a heavily glycosylated protein, the deglycosylated polypeptide chain comprising only 35 kDa. The glycosylated protein contains N-acetyl-D-glucosamine and sialic acid, but no N-acetyl-D-galactosamine. Glycosylation is responsible for substantial heterogeneity in the receptor on phorbol ester-stimulated U937 cells, and also for molecular weight variations among various cell lines. The amino acid composition and the NH2-terminal amino acid sequence are reported. The protein has a high content of cysteine residues. The NH2-terminal sequence is not closely related to any known sequence. The identification of the purified and sequenced protein with the human u-PA receptor is based on the following findings: 1) the ability of the purified protein to bind u-PA and its **amino-terminal fragment**; 2) the identical electrophoretic mobilities observed for cross-linked conjugates, formed between either the purified protein or the u-PA receptor on intact U937 cells and the above ligands; 3) the identity of the apparent molecular weight of the purified protein to that predicted for the u-PA receptor in the same cross-linking studies; 4) the identical extent of glycosylation of the purified protein and of the u-PA receptor in crude membrane fractions, as detected after cross-linking; 5) the ability of antibodies raised against the purified protein to inhibit cellular binding of the **amino-terminal fragment** of u-PA.

L17 ANSWER 100 OF 109 MEDLINE on STN

90201283. PubMed ID: 2156717. Localization of **urokinase**-type plasminogen activator receptor on U937 cells: phorbol ester PMA induces heterogeneity. Hansen S H; Behrendt N; Dano K; Kristensen P. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Experimental cell research, **(1990 Apr)** 187 (2) 255-62. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB The binding of human **urokinase**-type plasminogen activator (u-PA) to the surface of the human monocytic cell line U937 was studied by immunological detection of bound u-PA or binding of biotinylated diisopropyl fluorophosphate-inactivated human u-PA visualized by light or electron microscopy. Untreated U937 cells showed a characteristic binding pattern, with the majority of the u-PA bound to the microvillar-containing protruding pole of the cells. After treatment with the phorbol ester PMA, the resulting adherent cell population was very heterogeneous with respect to both cellular morphology and u-PA binding. The bound u-PA was distributed on both the dorsal and the substrate side of the cells, and the patches of bound u-PA could not be correlated to any typical membrane conformations or cell-cell or cell-substratum contacts. When a monoclonal antibody directed against the **amino-terminal fragment** (**ATF**) of u-PA was used, the results were identical regardless of whether intact u-PA or **ATF** was used for binding to the cells. In contrast, when a monoclonal antibody recognizing the non-receptor-binding protease domain of u-PA was used, bound **ATF** showed no staining, while bound intact u-PA

was stained as efficiently as above. The alteration of u-PA receptor distribution following treatment with PMA could be related to the changes in glycosylation and ligand affinity of the purified u-PA receptor previously described following PMA treatment of U937 cells.

L17 ANSWER 101 OF 109 MEDLINE on STN
90153917. PubMed ID: 2154462. Interaction of single-chain **urokinase**-type plasminogen activator with human endothelial cells. Barnathan E S; Kuo A; Rosenfeld L; Kariko K; Leski M; Robbiati F; Nolli M L; Henkin J; Cines D B. (Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia 19104.) Journal of biological chemistry, **(1990 Feb 15)** 265 (5) 2865-72. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB The interaction of **urokinase**-type plasminogen activators with receptors on the surface of endothelial cells may play an important role in the regulation of fibrinolysis and cell migration. Therefore, we investigated whether human umbilical vein endothelial cells (HUVEC) express receptors for single-chain **urokinase** (scu-PA) on the cell surface and examined the effect of such binding on plasminogen activator activity. Binding of 125I-labeled scu-PA to HUVEC, performed at 4 degrees C, was saturable, reversible, and specific (k+1 4 +/- 1 X 10(6) min-1 M-1, k-1 6.2 +/- 1.4 X 10(-3) min-1, Kd 2.8 +/- 0.1 nM; Bmax 2.2 +/- 0.1 X 10(5) sites/cell; mean +/- S.E.). Binding of radiolabeled scu-PA was inhibited by both natural and recombinant wild-type scu-PA, high molecular weight two-chain u-PA (tcu-PA), catalytic site-inactivated tcu-PA, an **amino-terminal fragment** of u-PA (amino acids 1-143), and a smaller peptide (amino acids 4-42) corresponding primarily to the epidermal growth factor-like domain. Binding was not inhibited by low molecular weight **urokinase** or by a recombinant scu-PA missing amino acids 9-45. Cell-bound scu-PA migrated at its native molecular mass on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the presence of plasminogen, scu-PA bound to endothelial cells generated greater plasmin activity than did scu-PA in the absence of cells. In contrast, when tcu-PA was added directly to HUVEC, sodium dodecyl sulfate-stable complexes formed with cell or matrix-associated plasminogen activator inhibitors with a loss of plasminogen activator activity. These studies suggest that endothelial cells in culture express high affinity binding sites for the epidermal growth factor domain of scu-PA. Interaction of scu-PA with these receptors may permit plasminogen activator activity to be expressed at discrete sites on the endothelial cell membrane.

L17 ANSWER 102 OF 109 MEDLINE on STN
90085165. PubMed ID: 2480654. Epitope mapping of the anti-**urokinase** monoclonal antibody 5B4 by isolated domains of **urokinase**. Corti A; Sarubbi E; Soffientini A; Nolli M L; Zanni A; Galimberti M; Parenti F; Cassani G. (Merrell Dow Research Institute, Lepetit Research Center, Gerenzano (Varese), Italy.) Thrombosis and haemostasis, **(1989 Nov 24)** 62 (3) 934-9. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB The **amino terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA) is a degradation product comprising the entire growth factor-like and kringle domains. It has been previously shown that **ATF** is able to bind to the u-PA receptor through the growth factor-like domain and that the anti u-PA monoclonal antibody 5B4 (Mab 5B4) binds to **ATF** preventing u-PA receptor binding. To localize more precisely the epitope recognized by Mab 5B4, **ATF** was subfragmented by controlled enzymatic proteolysis with V8 protease. Three subfragments of 4,000 Mr (F-4k), 11,000 Mr (F-11k) and 12,000 Mr (F-12k) were purified from the reaction mixture and characterized. SDS-PAGE under reducing and non-reducing conditions, N-terminal aminoacid sequence analysis and C-terminal aminoacid analysis of each fragment indicate that F-4k and F-11k correspond to intact growth factor-like domain and kringle domain (residues 4-43 and 44-135 respectively) while F-12k corresponds to the

kringle domain cleaved in the first loop at the glu52-gly53 bond. By Western blot and competitive binding experiments we show that Mab 5B4 recognizes an epitope located on the kringle domain of u-PA and that the binding is strongly reduced when the kringle contains an additional cleavage in its first loop. Since the receptor binding site of u-PA has been previously shown to be located on the growth factor-like domain, Mab 5B4 inhibits the binding of uPA to its cellular receptor likely by steric hindrance. Besides the proven utility in epitope localization of anti u-PA monoclonal antibodies, these u-PA fragments may represent powerful tools for studies of structure-function relationship of u-PA.

L17 ANSWER 103 OF 109 MEDLINE on STN

89296897. PubMed ID: 2544876. Accessibility of receptor-bound **urokinase** to type-1 plasminogen activator inhibitor. Cubellis M V; Andreasen P; Ragno P; Mayer M; Dano K; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Proceedings of the National Academy of Sciences of the United States of America, **(1989 Jul)** 86 (13) 4828-32. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB **Urokinase** plasminogen activator (uPA) interacts with a surface receptor and with specific inhibitors, such as plasminogen activator inhibitor type 1 (PAI-1). These interactions are mediated by two functionally independent domains of the molecule: the catalytic domain (at the carboxyl terminus) and the growth factor domain (at the amino terminus). We have now investigated whether PAI-1 can bind and inhibit receptor-bound uPA. Binding of 125I-labeled **ATF** (**amino-terminal fragment** of uPA) to human U937 monocyte-like cells can be competed for by uPA-PAI-1 complexes, but not by PAI-1 alone. Performed 125I-labeled uPA-PAI-1 complexes can bind to uPA receptor with the same binding specificity as uPA. PAI-1 also binds to, and inhibits the activity of, receptor-bound uPA in U937 cells, as shown in U937 cells by a caseinolytic plaque assay. Plasminogen activator activity of these cells is dependent on exogenous uPA, is competed for by receptor-binding diisopropyl fluorophosphate-treated uPA, and is inhibited by the addition of PAI-1. In conclusion, in U937 cells the binding to the receptor does not shield uPA from the action of PAI-1. The possibility that in adherent cells a different localization of PAI-1 and uPA leads to protection of uPA from PAI-1 is to be considered.

L17 ANSWER 104 OF 109 MEDLINE on STN

89123287. PubMed ID: 2521625. Plasminogen activation initiated by single-chain **urokinase**-type plasminogen activator. Potentiation by U937 monocytes. Ellis V; Scully M F; Kakkar V V. (Thrombosis Research Unit, King's College School of Medicine and Dentistry, London, United Kingdom.) Journal of biological chemistry, **(1989 Feb 5)** 264 (4) 2185-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The binding of **urokinase**-type plasminogen activators (u-PA) to receptors on various cell types has been proposed to be an important feature of many cellular processes requiring extracellular proteolysis. We have investigated the effect of single-chain u-PA binding to the monocyte-like cell line U937 on plasminogen activation. A 16-fold acceleration of the activation of plasminogen was observed at optimal concentrations of single-chain u-PA. This potentiation was abolished by the addition of either 6-aminohexanoic acid or the **amino-terminal fragment** of u-PA, thus demonstrating the requirement for specific binding of both single-chain u-PA and plasminogen to the cells. The mechanism of the enhancement of plasmin generation appears to be due primarily to an increase in the rate of feedback activation of single-chain u-PA to the more active two-chain u-PA by cell-bound plasmin, initially generated by single-chain u-PA. This increased activity of the plasminogen activation system in the presence of U937 cells provides a mechanism whereby u-PAs may exert their influence in a variety of cell-associated proteolytic events.

L17 ANSWER 105 OF 109 MEDLINE on STN

88115381. PubMed ID: 2828365. A 55,000-60,000 Mr receptor protein for **urokinase**-type plasminogen activator. Identification in human tumor cell lines and partial purification. Nielsen L S; Kellerman G M; Behrendt N; Picone R; Dano K; Blasi F. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of biological chemistry, **(1988 Feb 15)** 263 (5) 2358-63. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The iodinated Mr approximately equal to 15,000 **amino-terminal fragment** (**ATF**) of the **urokinase**-type plasminogen activator (u-PA) molecule bound specifically to the cell surface of all of seven cultured human tumor cell lines studied. Cross-linking of iodinated **ATF** to the cell surface using a bifunctional amino-reactive reagent followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography revealed with the four cell lines studied the occurrence of a single band migrating with an Mr of 70,000-75,000, indicating complex formation with an Mr of 55,000-60,000 u-PA receptor protein (u-PA-R). In the human monocyte cell line U937 cultivated in the presence of phorbol ester, the amount of complex was strongly increased, and a fraction of the complex had a slower electrophoretic mobility. Comparison between autoradiograms of reduced and unreduced samples suggests that u-PA-R consists of one polypeptide chain. Two forms of u-PA-R, which differed with respect to affinity to concanavalin A, were identified. u-PA-R retained its ability to bind to **ATF** after cell lysis, and it was purified approximately 2,200-fold from biosynthetically labeled U937 cells by affinity chromatography with proenzyme u-PA coupled to Sepharose. The purified Mr 55,000-60,000 protein was specifically bound and cross-linked to u-PA, proenzyme u-PA, and **ATF**, but not to tissue-type plasminogen activator or other unrelated proteins.

L17 ANSWER 106 OF 109 MEDLINE on STN

88089216. PubMed ID: 3121772. Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. Grondahl-Hansen J; Agerlin N; Munkholm-Larsen P; Bach F; Nielsen L S; Dombernowsky P; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of laboratory and clinical medicine, **(1988 Jan)** 111 (1) 42-51. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB An enzyme-linked immunosorbent assay (ELISA) was developed for the measurement of human **urokinase**-type plasminogen activator (u-PA) in plasma and serum. Microtiter plates were coated with a monoclonal antibody and incubated with standard or sample. Bound u-PA was quantitated with polyclonal antibodies conjugated with biotin, followed by avidin-peroxidase. The assay was 10 times as sensitive as previously reported immunoassays, the detection limit being approximately 1 pg u-PA in a volume of 100 microliter, with a linear dose-response up to 15 pg u-PA. The assay detected active u-PA and its inactive proenzyme form equally well, and the recovery of both forms was higher than 90% in plasma. It also detected u-PA complexed with plasminogen activator inhibitor type 1. Various structurally related proteins, including t-PA, were tested, but no reaction was observed with proteins other than u-PA and its **amino-terminal fragment**. The intra-assay and interassay coefficients of variation for determination of u-PA in plasma were 7.6% and 8.4%, respectively. The ELISA was used to measure the concentration of u-PA in plasma from 34 healthy donors and 92 patients with breast cancer with a varying extent of disease. The mean value for the healthy donors was 1.1 +/- 0.3 ng/ml (SD) of u-PA in plasma. This value is substantially lower than those previously reported. The mean value for the patients with breast cancer was 1.3 +/- 0.4 ng/ml. This moderate increase was statistically significant at the 1% level. Approximately one quarter of the patients had plasma u-PA concentrations above the range

observed for the healthy controls. There was a positive correlation between the mean u-PA plasma concentration and the extent of disease in different groups of patients.

L17 ANSWER 107 OF 109 MEDLINE on STN

87165839. PubMed ID: 3031025. The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. Appella E; Robinson E A; Ullrich S J; Stoppelli M P; Corti A; Cassani G; Blasi F. Journal of biological chemistry, **(1987 Apr 5)** 262 (10) 4437-40. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Previous studies have shown that the region of human **urokinase**-type plasminogen activator (uPA) responsible for receptor binding resides in the **amino-terminal fragment** (**ATF**, residues 1-135) (Stoppelli, M.P., Corti, A., Soffientini, A., Cassani, G., Blasi, F., and Assoian, R.K. (1985) Proc. Natl. Acad. Sci. U.S. A. 82, 4939-4943). The area within **ATF** responsible for specific receptor binding has now been identified by the ability of different synthetic peptides corresponding to different regions of the amino terminus of uPA to inhibit receptor binding of 125I-labeled **ATF**. A peptide corresponding to human [Ala19]uPA-(12-32) resulted in 50% inhibition of **ATF** binding at 100 nM. Peptides uPA-(18-32) and [Ala13]uPA-(9-20) inhibit at 100 and 2000 microM, respectively. The human peptide uPA-(1-14) and the mouse peptide [Ala20]uPA-(13-33) have no effect on **ATF** receptor binding. This region of uPA is referred to as the growth factor module since it shares partial amino acid sequence homology (residues 14-33) to epidermal growth factor (EGF). Furthermore, this region of EGF is responsible for binding of EGF to its receptor (Komoriya, A. Hortsch, M., Meyers, C., Smith, M., Kanety, H., and Schlessinger, J. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 1351-1355). However, EGF does not inhibit **ATF** receptor binding. Comparison of the sequences responsible for receptor binding of uPA and EGF indicate that the region of highest homology is between residues 13-19 and 14-20 of human uPA and EGF, respectively. In addition, there is a conservation of the spacings of four cysteines in this module whereas there is no homology between residues 20-30 and 21-33 of uPA and EGF. Thus, residues 20-30 of uPA apparently confer receptor binding specificity, and residues 13-19 provide the proper conformation to the adjacent binding region.

L17 ANSWER 108 OF 109 MEDLINE on STN

87121003. PubMed ID: 2433787. A monoclonal antibody that recognizes the receptor binding region of **human urokinase plasminogen activator**. Nolli M L; Corti A; Soffientini A; Cassani G. Thrombosis and haemostasis, **(1986 Oct 21)** 56 (2) 214-8. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB An anti-**urokinase** monoclonal antibody 5B4 (MAB 5B4) was obtained by fusing the murine myeloma cell line X63-Ag8.653 with the spleen cells from a female BALB/c mouse immunized with **high-molecular-weight urokinase** (**HMW-uPA**). MAB 5B4 is an IgG1 that binds selectively to the single-chain form of uPA (sc-uPA), to **HMW-uPA** and to the 17,000 Mr aminoterminal fragment of the A-chain (**ATF**) but not to the low-molecular-weight **urokinase** (LMW-uPA) nor to the reduced form of **HMW-uPA**. This strongly suggests that MAB 5B4 recognizes a conformational determinant on the A-chain. The antibody has an affinity constant for uPA-Sepharose of 1.42 X 10(7) M-1, calculated from equilibrium binding data, and can be used for one step purification of **HMW-uPA** by immunoaffinity chromatography. MAB 5B4 and the previously obtained antibody 105IF10 recognize the A-chain: the epitopes, however, are distinct as shown by double-antibody-sandwich enzyme immunoassay. Finally MAB 5B4 inhibits the binding of **ATF** to the uPA receptor of different human cells, whereas 105IF10 does not. Thus this antibody represents a potentially, useful tool for the study of uPA receptor

physiology.

L17 ANSWER 109 OF 109 MEDLINE on STN
85270442. PubMed ID: 2991901. Differentiation-enhanced binding of the **amino-terminal fragment** of **human urokinase plasminogen activator** to a specific receptor on U937 monocytes. Stoppelli M P; Corti A; Soffientini A; Cassani G; Blasi F; Assoian R K. Proceedings of the National Academy of Sciences of the United States of America, **(1985 Aug)** 82 (15) 4939-43. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The purified **amino-terminal fragment** (**ATF**) of **human urokinase plasminogen activator** (residues 1-135), which is not required for activation of plasminogen, binds with high affinity to specific plasma membrane receptors on U937 monocytes. Intact **urokinase** efficiently competes for 125I-labeled **ATF** binding; 50% competition occurs with 1 nM **urokinase**. A large part of receptor-bound **urokinase** remains on the cell surface for at least 2 hr at 37 degrees C. Differentiation of U937 monocytes into macrophage-like cells specifically increases **ATF** binding 10- to 20-fold. These results suggest an important role for **urokinase** in monocyte/macrophage biology: the native enzyme binds to the cells with the amino-terminal domain; the catalytic, carboxyl-terminal domain remains exposed on the cell surface to stimulate localized proteolysis and facilitate cell migration.

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(FILE 'HOME' ENTERED AT 14:08:08 ON 18 AUG 2005)

FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005
L1 9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L2 231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3 106 S L2 AND AD<MAR 01 2001
L4 8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)
L5 25 S L3 AND POWDER
L6 19 S L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L7 1 S L4 AND L6
L8 1 S L7 AND (POWDER)
L9 1 S L8 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L10 1 S L9 AND PHARMACEUTICAL
L11 1 S L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)
L12 18 S L5 AND LYOPHILIZ?
L13 13 S L12 AND (TRANSNASAL OR INTRANASAL OR NASAL)
L14 13 S L13 AND PHARMACEUTICAL?

FILE 'USPATFULL' ENTERED AT 15:16:41 ON 18 AUG 2005

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L15 9749 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L16 147 S L15 AND (ATF OR AMINO TERMINAL FRAGMENT)
L17 109 S L16 AND (1985-2001/PY)

=> s l16 and (HIV or human immunodeficiency virus)
150299 HIV
1277471 HUMAN
119328 IMMUNODEFICIENCY
398362 VIRUS
46524 HUMAN IMMUNODEFICIENCY VIRUS
(HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L18 2 L16 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

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L18 ANSWER 1 OF 2 MEDLINE on STN

2003517119. PubMed ID: 12960238. The role of **urokinase**-type plasminogen activator (uPA)/uPA receptor in **HIV**-1 infection. Alfano Massimo; Sidenius Nicolai; Blasi Francesco; Poli Guido. (Department of Immunology and Infectious Disease, Vita-Salute University School of Medicine, Milan, Italy.) Journal of leukocyte biology, (2003 Nov) 74 (5) 750-6. Electronic Publication: 2003-08-21. Ref: 82. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB The binding of **urokinase**-type plasminogen activator (uPA) to its glycosyl-phosphatidyl-inositol (GPI) anchored receptor (uPAR) mediates a variety of functions in terms of vascular homeostasis, inflammation and tissue repair. Both uPA and uPAR, as well as their soluble forms detectable in plasma and other body fluids, represent markers of cancer development and metastasis, and they have been recently described as predictors of **human immunodeficiency virus** (**HIV**) disease progression, independent of CD4+ T cell counts and viremia. A direct link between the uPA/uPAR system and **HIV** infection was earlier proposed in terms of cleavage of gp120 envelope by uPA. More recently, a negative regulatory effect on both acutely and chronically infected cells has been linked to the noncatalytic portion of uPA, also referred to as the **amino-terminal fragment** (**ATF**). **ATF** has also been described as a major CD8+ T cell soluble **HIV** suppressor factor. In chronically infected promonocytic U1 cells this inhibitory effect is exerted at the very late stages of the virus life cycle, involving virion budding and entrapment in intracytoplasmic vacuoles, whereas its mechanism of action in acutely infected cells remains to be defined. Since uPAR is a GPI-anchored receptor it requires association with a signaling-transducing component and different partners, which include CD11b/CD18 integrin and a G-protein coupled receptor homologous to that for the bacterial chemotactic peptide formyl-methionyl-leucyl-phenylalanine. Which signaling coreceptor(s) is(are) responsible for uPA-dependent anti-**HIV** effect remains currently undefined.

L18 ANSWER 2 OF 2 MEDLINE on STN

2001327647. PubMed ID: 11394884. **Amino-terminal fragment** of **urokinase**-type plasminogen activator inhibits **HIV**-1 replication. Wada M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10 Murotani, Nishi-ku, Kobe, 651-2241, Japan.. wada-m@jcrpharm.co.jp) . Biochemical and biophysical research communications, (2001 Jun 8) 284 (2) 346-51. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB CD8+ T lymphocytes have been shown to produce unidentified soluble factors active in suppressing **HIV**-1 replication. In this study, we purified an **HIV**-1 suppressing activity from the culture supernatant of an immortalized CD8+ T cell clone, derived from an **HIV**-1 infected long-term nonprogressor, and identified this activity as the **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA). **ATF** is catalytically inactive, but suppresses the release of viral particles from the **HIV**-1 infected cell lines via binding to its receptor CD87. In contrast, cell proliferation and the secretion of an **HIV**-1 LTR driven reporter gene product were not affected by **ATF**. These findings suggest that **ATF** may inhibit the assembly and budding of **HIV**-1, which provides a novel therapeutic strategy for AIDS.

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(FILE 'HOME' ENTERED AT 14:08:08 ON 18 AUG 2005)

FILE 'USPATFULL' ENTERED AT 14:08:17 ON 18 AUG 2005

L1 9253 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL

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L2          231 S L1 AND (AMINO TERMINAL FRAGMENT OR ATF)
L3          106 S L2 AND AD<MAR 01 2001
L4            8 S L3 AND (ATF/CLM OR AMINO TERMINAL FRAGMENT/CLM)
L5           25 S L3 AND POWDER
L6           19 S L5 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L7            1 S L4 AND L6
L8            1 S L7 AND (POWDER)
L9            1 S L8 AND (LECITHIN OR METHYLCELLULOSE OR MANNITOL OR LACTOSE)
L10           1 S L9 AND PHARMACEUTICAL
L11           1 S L10 AND (TRANSNASAL OR INTRANASAL OR NASAL)
L12          18 S L5 AND LYOPHILIZ?
L13          13 S L12 AND (TRANSNASAL OR INTRANASAL OR NASAL)
L14          13 S L13 AND PHARMACEUTICAL?

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     FILE 'MEDLINE' ENTERED AT 15:16:44 ON 18 AUG 2005
L15        9749 S (UROKINASE OR HIGH MOLECULAR WEIGHT UROKINASE OR HIGH MOLECUL
L16         147 S L15 AND (ATF OR AMINO TERMINAL FRAGMENT)
L17         109 S L16 AND (1985-2001/PY)
L18           2 S L16 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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STN INTERNATIONAL LOGOFF AT 15:31:26 ON 18 AUG 2005
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